Title

Fluorinated α-amino acid analogues of L-methionine and related compounds for use as potential enzyme inhibitors

Author: Bernadette Johnson

This thesis is submitted to the Physical Sciences Graduate School in part fulfilment of the requirements for completing a Post higher Degree at the University of Glasgow.

The research was conducted in the Department of Chemistry, Joseph Black Building, University of Glasgow.

August 1998

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

This thesis is dedicated to my mother Sara and my husband Alister for the support and understanding they have always shown to me. I would not be where I am today without the two of you. Many thanks.

I love you both
Bernadette

Acknowledgements

Dr. Ernest. W. Colvin

Prof. Graham. H. Coombs

David Laughland

All the Technical Staff at the Chemistry Dept.

Loudon Bequest Fund

Sullom Voe Participants 10th Anniversary Education Scholarship Fund

Abbreviations

AIDS	Acquired Immuno Defficiency Syndrome
Boc	Butoxycarbonyl
b.p.	Boiling point
$Conc^n$	Concentration
D	Dextro
DAST	Diethylaminosulfur trifluoride
DCM	Dichloromethane
DNA	Deoxyribonucleic acid
EtOAc	Ethyl acetate
Et_2O	Diethyl ether
EtOH	Ethanol
Expt	Experiment
Fmoc	9-Fluorenylmethylcarbonyl
g	Grams
h	Hour
IR	Infra red
HIV	Human Immuno-deficiency Virus
Hz	Hertz
KOH	Potassium hydroxide
L	Levo
M	Mole
MCPBA	m-Chloroperbenzoic acid
MeOH	Methanol
$MeNO_2$	Nitromethane
MHz	Mega Hertz
Min	Minute
mgs	Milligrams
ml	Millilitre
mmol	Millimoles
m.p.	Melting point
m/z	mass of the ion
NAD	Nicotinamide adenine dinucleotide
NaOH	Sodium hydroxide
NMR	Nuclear Magnetic Resonance
PLP	Pyridoxal phosphate
ppm	Parts per million
PTFE	Poly(tetrafluoroethylene)
RDD	Rational Drug Design
SET	Single Electron Transfer
S_{RN}	Substitution Radical Nucleophilic
TLC	Thin Layer Chromatography
U.V.	Ultra Violet

Summary

With the discovery of AIDS (and the HIV virus) in the 1970's it has become increasingly apparent that AIDS and other immuno-compromised patients are particularly vulnerable to viral and fungal infections. If left untreated these infections which may only cause minor discomfort in a healthy individual can ultimately lead to the death of the immuno-compromised patient. The disease trichomoniasis found in humans and caused by the anaerobic flagellate protozoa *Trichomonas vaginalis* is one such infection.

In the past trichomonal infections, including trichomoniasis, have been treated using 5-nitroimidazoles. However these agents can give rise to resistant strains of the parasite and are suspected of having mutagenic effects. The drawbacks of these existing treatments demonstrate the need to identify new pro-drugs of greater specificity towards the disease processes of viral and fungal infections. In the case of the organism *Trichomonas vaginalis,* we already know that the organism contains the enzyme L-methionine-γ-lyase and that this enzyme performs a function peculiar to *T. vaginalis*. This peculiarity is a potential source of increased specificity for this organism's particular disease process and as such the enzyme L-methionine-γ-lyase presents itself as a target for therapeutic attack. If the suggested role of the enzyme is correct, then specific inhibitors of L-methionine-γ-lyase could significantly reduce or even cure the pathogenicity of *T. vaginalis.*

In recent years a large number of compounds have been found to function as enzyme activated suicide substrates, through loss of a halide or other good leaving group. The vigorous development of organofluorine chemistry together with further advances in the understanding of biochemical processes have led to an increased level of interest and research in the area of fluorine containing

aminoacids. Many fluorinated amino acids are now proving to be highly selective inhibitors of pyridoxal-5'-phosphate dependent enzymes.

Following a rational drug design (RDD) approach, it was our aim to synthesise potential anti-metabolites for the therapeutic treatment of infections arising from anaerobic micro-organisms and helminths, in particular focusing our attention on the treatment of infections arising from the anaerobic flagellate protozoa *T. vaginalis.*

The sulfur containing amino acid L-methionine plays diverse biochemical and physiological roles not only as a constituent of peptides and proteins but also as a precursor in the biosynthesis of the DNA methylating agent 5-adenosylmethionine. In addition to its importance in both biochemical and physiological processes, L- methionine is a natural substrate for L-methionine-γ-lyase. Our experimental starting point has been to try to exploit this enzyme-substrate binding specificity. To do this we synthesised and biologically tested fluorinated analogues of L-methionine for growth inhibiting properties and or selective toxicity in *T. vaginalis,* an organism known to contain L-methionine-γ-lyase.

In addition and for comparison with L-trifluoromethionine we undertook to synthesise and biologically test the trifluoromethyl analogues of L-cysteine and D-penicillamine.

In an alternative attempt to identify a selectively toxic compound for *T. vaginalis* we also explored a mechanism based enzyme inactivation approach involving the 2,3-sigmatropic rearrangement of an allyl sulfoxide to an allyl sulfenate ester in the enzyme active site. Via this approach the allyl sulfenate ester situated in the enzyme active site serves to derivatise and deactivate the target enzyme. This approach required the synthesis of 2-amino-4-chloro-5-(*p*-tolylsulfinyl) pentanoic acid from allylglycine. In an effort to extend our chemical and biological understanding of this alternative approach we also attempted to synthesise

analogous chloro, fluoromethyl (sulfinyl) pentanoic acid compounds. It was hoped that these new compounds would create more favourable equilibrium concentrations of the sulfenate ester in the active site and that the presence of fluorine would convey an increased level of toxicity through improved lipophilicity and susceptibility to nucleophilic attack.

Contents

INTRODUCTION

Chapter 1

The Discovery of the Role of Micro-organisms in the Causation of Disease

1.1 Human disease

A disease is a condition that impairs the proper function of the body or of one of its parts. Every living thing, both plants and animals, can succumb to disease. Hundreds of different diseases exist. Each has its own particular set of symptoms and signs that enable a diagnosis. Every disease has a cause, although the cause of some diseases still remains to be discovered. Every disease displays a cycle of onset, course of affliction and end, when it disappears or it partially disables or kills its victim.

As far back as history can be traced, including Egyptian hieroglyphics, there have been references made to medicinal remedies for the treatment of various diseases (1).

1.2 Micro-organisms as agents of infectious disease

Microbes are ubiquitous organisms, they are found in the soil, the air and our own bodies. In most individuals they can co-exist without causing any harm to

their unwitting host. However as we now know microbes can act as agents of infectious disease.

The discovery of the role of micro-organisms in the causation of disease was not made until Louis Pasteur's studies on fermentation, carried out between 1857 - 1876. Ever conscious of the practical applications of his work, he devoted considerable attention to the spoilage of beer and wine, demonstrating that spoilage was a result of the growth of undesirable micro-organisms. Pasteur used a peculiar and significant term to describe these microbial induced spoilage processes, he called them "diseases" of beer and wine. In fact he was already considering the possibility that micro-organisms may act as agents of infectious disease in higher organisms. Some evidence in support of his hypothesis already existed. It had been shown in 1813 that a specific fungus caused diseases of wheat and rye and in 1845 M.J. Berkeley had proven that the great potato blight of Ireland, a natural disaster that deeply influenced Irish history, was caused by a fungus (2).

The first recognition that fungi may be specifically associated with a disease of animals came in 1836 through the work of A. Bassi in Italy on fungal diseases in silkworms. A few years later J.L. Schönlein showed certain skin diseases in man were caused by fungal infections. Despite these indications, very few medical scientists were willing to entertain the notion that major infectious diseases in man could be caused by micro-organisms, and fewer still believed that organisms as small and apparently simple as the bacteria could act as agents of disease.

Some years later a young British surgeon, J. Lister, who was deeply impressed by the work of Pasteur, reasoned that surgical sepsis, a serious problem of the day, might well result from microbial infections of the tissue exposed during operation. Around 1864 Lister developed procedures of antiseptic surgery and although these procedures were initially greeted with considerable scepticism, once their success in the prevention of surgical sepsis was widely recognised

they eventually became common practice. This discovery provided powerful, albeit indirect, evidence for the 'germ theory' of disease.

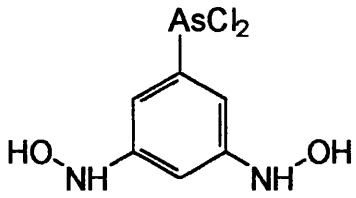
The breakthrough in linking bacteria as specific agents of infectious disease in animals was made during the study of anthrax, a serious infection of domestic animals that is transmissible to humans. R. Koch, a German country doctor, demonstrated conclusively in 1876 that mice could be infected with material from a diseased domestic animal. He transmitted the disease through a series of 20 mice by successive inoculation; at each transfer the characteristic symptoms were observed. He then proceeded to cultivate the bacterium by introducing minute heavily infected particles of spleen from a diseased animal into drops of sterile serum. Observing hour after hour the growth of the micro-organisms in this culture medium he saw the rods change into long filaments within which ovoid, refractile bodies eventually appeared, now know as *Bacillus anthracis* (2).

Koch carried out another series of experiments that demonstrated the biological specificity of disease agents. He showed that another spore forming bacterium, the hay bacillus, does not cause the disease anthrax upon injection. From this and other studies he concluded that only one kind of bacillus is able to cause the specific disease process anthrax, while other bacteria either do not produce disease following inoculation or give rise to other kinds of disease. This work marked the start of the golden age of medicinal bacteriology (3).

1.3 Control of bacteriological disease

The importance of acquiring immunity as a means of protection against specific bacteriological diseases was recognised shortly after the discovery of the role of micro-organisms as the agents of infectious disease. For several decades thereafter (from the late 1880's to the early 1900's) control of infectious disease was based exclusively on the use of antisera and vaccines, and was largely preventative. Usually little could be done to cure an infection once it appeared.

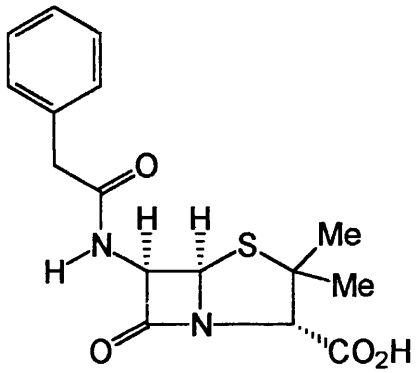
A different kind of approach to the control of infectious disease was developed in 1908 by the German physician-chemist P. Erhlich. Erhlich initiated an empirical search for synthetic chemicals that possess selective toxicity for pathogenic micro-organisms. He coined the word 'chemotherapy' to describe this approach. A few years earlier, in 1905, H. Thompson discovered that an arsenic containing organic compound 'atoxyl' was effective in treating trypanosomiasis, a protozoal disease common in parts of Africa and South America. Erhlich set out to modify atoxyl systematically in the hope of finding a compound which would be selectively toxic to pathogens. His efforts produced one success; *Salvarsan* (arsphenamine) which proved to be effective in the treatment of syphilis, caused by the protozoa *Spirochaeta pallida*, and other spirochaetal infections. Even though *Salvarsan* produced severe side effects, it remained the only treatment of spirochaetal infections and the only example of chemotherapy of infectious disease until the late 1920's and the introduction of antibiotics (4).



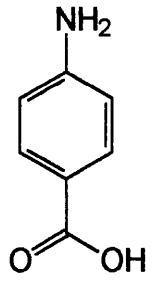
arsphenamine *(Salvarsan)*

The years between 1928 and 1940 were the most fruitful in the discovery and development of anti-microbial drugs. In 1928 a British scientist, Sir Alexander Fleming, noticed that a mold growing in one of his laboratory cultures was able to destroy that culture's microbes. Since the mold was a species of *Penicillium*, he named the germ-killing substance penicillin. In 1941 and thanks to the pioneering efforts of Florey and coworkers, penicillin was used to treat serious infections. The results were dramatic with patients who received the drug making rapid and complete recoveries (5).

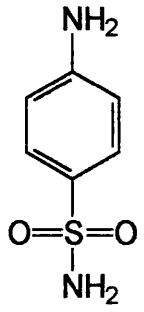
The next significant advance in chemotherapy came in 1935 and was also made empirically. Large numbers of aniline dyes were screened for anti-bacterial chemotherapeutic activity. In this class of compounds one substance *Prontosil,* discovered by G. Domagk, was found to be effective. Its anti-bacterial activity in infected animals was attributed to a colourless breakdown product sulfanilamide, formed in the animal's body. D.D. Woods observed that the inhibition of bacterial growth by sulfanilamide can be reversed by the structural analogue p-aminobenzoic acid. Woods deduced that p-aminobenzoic acid is both a normal constituent of the bacterial cell and also a biosynthetic precursor of the coenzyme folic acid. While mammals obtain folic acid from dietary sources, most bacteria synthesise folic acid from p-aminobenzoic acid. Sulfanilamide blocks the conversion of p-aminobenzoic acid to folic acid by 'tricking' the bacteria into using it instead of the close structural relation p-aminobenzoic acid that it really needs. In this way sulfanilamide is selectively toxic to bacteria (4).



penicillin



p-aminobenzoic acid.

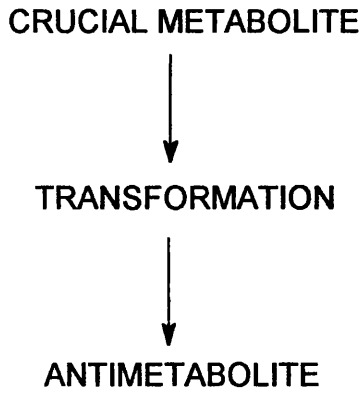


Sulfanilamide

1.4 The modern era of medicinal chemistry

Woods' work in the 1930's and 1940's on sulfanilamide appeared to offer a rational approach to chemotherapy through the synthesis of analogues of known essential metabolites. Woods' work marks the beginning of the modern era of medicinal chemistry and a shift in the medicinal chemist's attention from endogenous metabolites to the biochemical processes which control the flux of metabolites and to the rational drug design approach.

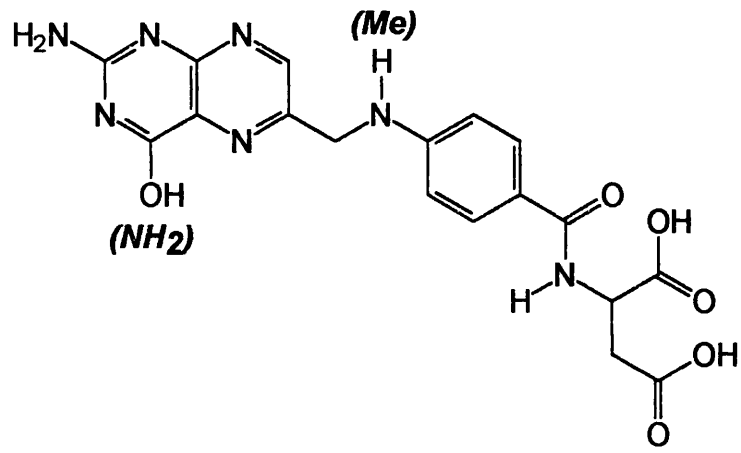
The first step in a rational drug design process is the careful selection of a crucial metabolite of a key biological process believed to be critical to a "disease process". The second step is to effect the conversion of the crucial metabolite into an anti-metabolite, that is into a compound which will as closely as possible mimic the metabolite whilst at the same time be different enough to effect inhibition of the key chemical or physico-chemical process.



Summary of the rational drug design approach

Many of the important biochemical processes are enzyme catalysed reactions of biosynthesis and catabolism. In the years since Woods' work on sulfanilamide thousands of structural analogues of amino acids, purines, pyrimidines and vitamins have been synthesised and tested and a number of useful chemotherapeutic agents discovered. One such example is methotrexate discovered in the 1940's. Although the mechanism of action was not fully appreciated at the time of discovery, methotrexate, in its role as an

antimetabolite, inhibits cancer tumour growth by preventing DNA synthesis. As a structural analogue of folic acid, methotrexate acts as a competitive inhibitor of the enzyme dihydrofolate reductase and prevents the formation of the reduced folate required for DNA synthesis (6).



folic acid ***(methotrexate)***

In the following chapter we will concentrate our discussion on enzymes as targets for medicinal chemistry.

Chapter 2

Enzymes in Medicine

In the field of molecular sciences, enzyme chemistry occupies a special niche as one of the major contact points between the chemical and biological disciplines. The special properties of enzymes as selective and highly efficient catalysts are so central to current medical and chemical challenges that the development of enzyme chemistry over the past 40 years has been a major stimulus to chemical research in general.

2.1 Enzymes

Enzymes were already prompting a great deal of interest and chemical research in the first half of the 19th century. When we look back in the literature we find as early as 1833 observations had been made of the phenomenon of the natural hydrolysis of potato starch. At this time the idea that enzymes are

chemicals was treated with a great deal of scepticism and controversy. Two conflicting views were held. The first view, held by amongst others L. Pasteur, believed that enzymatic activity was a property inseparable from living cells. Pasteur believed that living yeast cells were necessary for the fermentation process, hence the name 'Enzyme' meaning yeast was born. The second and opposing view, held by the prominent chemists J. Liebig and F. Wöhler, regarded enzymes as chemical catalysts, which could be separated from cells. In 1897 Buchner settled the dispute by experiment. He demonstrated that a yeast extract was capable of sustaining the fermentation of sugar. A few years earlier in 1894 a series of papers published by E. Fischer discussed the stereoselectivity in enzyme catalysis (7). By experiment Fischer showed that the enzyme which hydrolyses sucrose, called 'invertin' by Fischer, acts only upon α-D-glucosides: β-D-glucosides and L-glucosides were completely untouched. This result along with other complementary experimental data proved conclusively that enzymes are discriminatory with respect to the configuration of their substrate. The ability of enzymes to select between stereoisomers still remains one of their most important and alluring properties.

The first steps in the synthetic use of enzymes were made when Croft-Hill demonstrated that yeast enzymes could be used synthetically and Emmerling reported the synthesis of amydalin using enzymes. So even at the turn of the century we knew many of the fundamental properties of enzymes.

2.1.1 What is an enzyme?

So what is an enzyme? This question was answered in the 1920's after the successful isolation of the enzyme urease by J.B. Sumner (8). The purification and crystallisation of this enzyme and others that followed showed clearly that enzymes are proteins. Since then our knowledge of enzymes has greatly increased and we now know they act as excellent catalysts in biochemical reactions, accelerating reactions by factors of at least a million. In many biological systems the reactions do not occur at perceptible rates in the

absence of enzymes. A survey of the different types of reaction catalysed by enzymes reveals that the common categories of organic and inorganic reactions apply.
These categories include for example:

i) substitution ii) addition iii) elimination iv) oxidation v) reduction

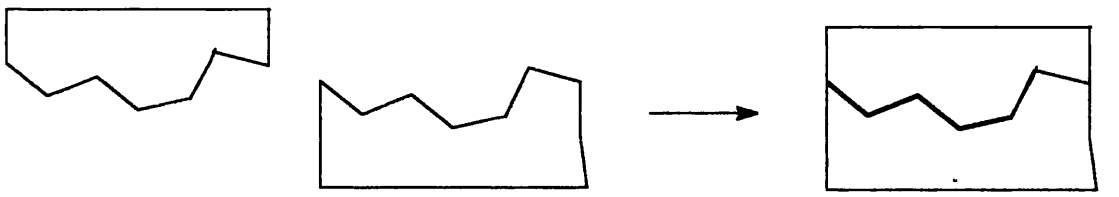
Although the enzyme-catalysed reaction types are very often obvious the mechanism by which they occur can often only be speculated since the structure of many enzymes are still unknown.

The mechanistic nature of enzyme chemistry has increased awareness in the principles of physical organic chemistry. The physical organic chemist is concerned with the properties of organic molecules in systematic relationship to their structure and reactivity. Enzyme chemistry is a logical extension of this interest since catalysis and specificity, the central features of enzyme reactions, are already very familiar concepts to the physical organic chemist.

Enzymes are much larger than any usual reaction substrate and as such provide the environment of the reaction. This environment is often referred to as the active site of the enzyme. The active site of an enzyme is a 3-dimensional entity formed by groups that come together from different parts of the amino acid sequence. The non-covalent binding in the enzyme-substrate complex involves irreversible interactions mediated by electrostatic interactions, hydrogen bonds, van der Waals forces and hydrophobic interactions. Active sites tend to be clefts or crevices and in order to fit into a cleft or crevice the substrate must have a matching shape. The biological activity of a substrate originates from the enzyme-substrate binding.
E. Fischer's analogy of a lock and key expressed in 1890 gives a good visual interpretation of enzyme substrate binding. This process of dynamic recognition is called 'induced fit'. Once bound a cascade of events amplifies the original molecular interaction via the biological activity of different factors such as steric hindirance, electronegativity, dipole interactions, lipophilicity etc. all of which contribute to the overall process of molecular recognition.

Schematic Representation of Enzyme-Substrate Binding

(Fischer's Lock and Key analogy)



Substrate	Enzyme Active Site	Enzyme-Substrate Binding
(key)	(lock)	(lock + key)

However it is now known that the physical rigidity of a lock and key do not appropriately describe the mobile enzyme-substrate interaction and that the active sites of some enzymes are modified by the binding of a substrate.

Enzymatic activity is influenced by:

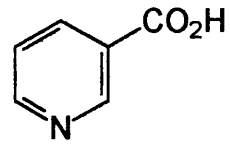
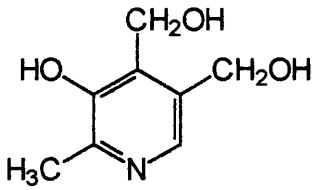
i) Substrate Concentration *ii) Temperature* *iii) pH*

each of which must lie within a certain range for the enzyme to function. Selectivity in catalysis, as Fischer surmised, is of immense importance in that it can be applied in a direct sense to perform both regioselective and stereoselective transformations in organic synthesis.

2.1.2 Enzymic reactions

Each enzyme is specific to a particular reaction or group of similar reactions and many enzymes will require the association of certain cofactors in order to function. A cofactor is the non-protein portion of the enzyme and can be a simple metal ion; for example copper ion is the cofactor for the enzyme ascorbic acid oxidase. Other enzymes contain non-protein organic molecules as cofactors. An organic prosthetic group is frequently referred to as a co-enzyme. Two examples are given below (table 1). If an organism cannot synthesis a necessary cofactor then the cofactor must be present in small amounts in the diet.

Table 1 - Examples of Co-enzymes

cofactor	*vitamin required by organism to provide cofactor*	*structure of vitamin*
NAD^+	nicotinic acid (niacin)	
pyridoxal phosphate	pyridoxine	

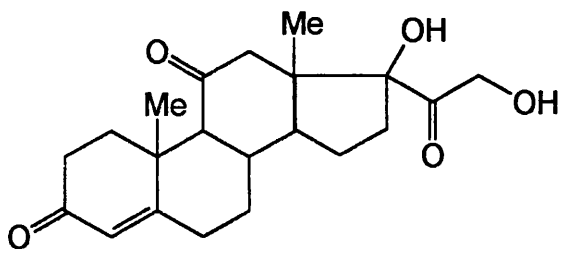
In effect enzymes catalyse reactions by stabilising transition states in reaction pathways. By doing this selectively an enzyme determines which of several potential chemical reactions actually occurs.

2.2 Enzymes as targets for drug design

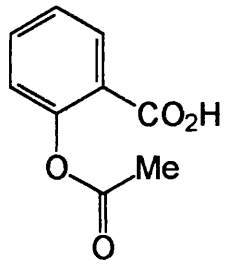
By elucidating the mechanism by which an enzyme catalyses a reaction we can further our understanding of how enzymes accomplish their tasks. With this knowledge we can either i) begin to design or modify molecules to become useful catalysts or ii) use enzymes themselves as catalysts for reactions outside their normal metabolic functions. It is this second possibility with which we will concern ourselves in the remainder of this chapter. In particular we will focus on the role of enzymes in the selective inhibition of key metabolic pathways and how this type of inhibition can be utilised in the treatment of medical conditions such as microbial and viral infections.

The history of medicinal chemistry is full of examples of drug searches in which recognition that the target at the molecular level was an enzyme came long after the drug was discovered. Often it was the case that a clear understanding of the biochemical mechanism of a particular disease only became possible after the drug was available. The discovery of the non-steroidal anti-inflammatory drugs (NSAIDs) is an excellent example of this. These drugs

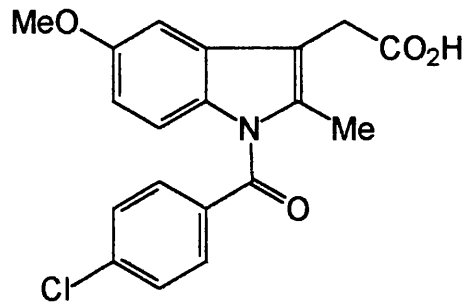
were the product of extensive research programmes aimed at alleviating rheumatoid arthritis through the use of steroids (rheumatoid arthritis is a chronic crippling disease of the connective tissue which deforms the bone joints). Aspirin had been used in the treatment of rheumatoid arthritis for about half a century before the introduction in the 1950s of the powerful anti-inflammatory steroids. These steroids, of which cortisone is an example, although highly effective as anti-inflammatory drugs had a tendency to induce hormonal and metabolic side effects and as such proved to be of only limited medical use. However the discovery of the anti-inflammatory steroids provided the stimulus in the scientific community to search for other compounds of novel structure with anti-inflammatory activity. These efforts led to the discovery of indomethacin in 1961 and shortly after ibuprofen. However it was not until 1971 that J.R. Vane (9) was able to demonstrate that both indomethacin and aspirin inhibit the biosynthesis of prostaglandins, some of which are important mediators of inflammation. Prostaglandins are produced from the metabolism of arachidonic acid and their production is controlled by the enzyme cyclo-oxygenase.



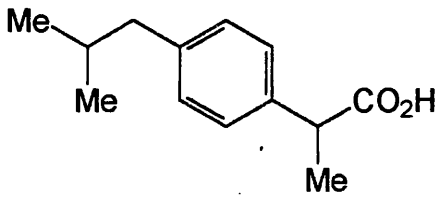
cortisone



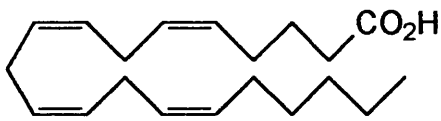
aspirin



indomethacin



ibuprofen



arachidonic acid

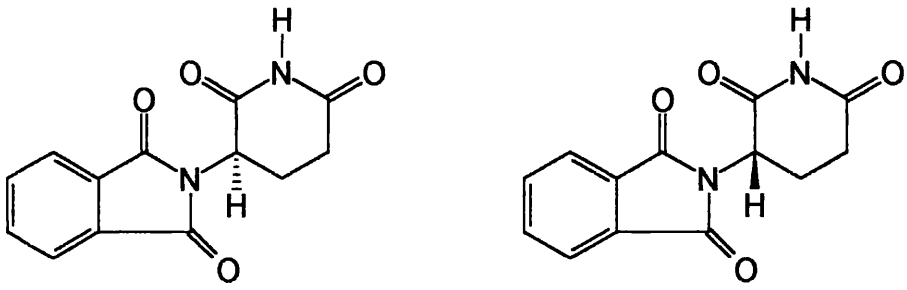
2.3 Substrate stereospecificity

For many years it was considered that the racemic form of any chiral compound should in the worst case have only half the activity of the more active enantiomer. It is only in the last 30 years that the scientific community and the drug regulatory authorities have fully recognised the relevance of chirality to biological activity and molecular recognition (10,11). It is now commonly accepted that there are often likely to be differences in the uptake and transportation of 2 complementary enantiomers. Two antipodes can also exhibit different affinities and / or intrinsic activities at receptor sites, which can result in different responses.

e.g. the historical example of thalidomide ($C_{13}H_{10}N_2O_4$)

(once referred to as the safest drug ever)

Between 1959 and 1962 thousands of deformed babies were born to West German and British mothers who had taken the sedative thalidomide while they were pregnant to alleviate morning sickness. It is now known that although the L-enantiomer of thalidomide acts as a sedative the D-enantiomer interferes with foetal growth and in particular formation of the arms and legs. (12)



(D) (L)

L and D enantiomers of thalidomide

It is a great challenge for chemists to understand the chemical basis for enzymatic catalysis and a still greater one to mimic it effectively.

2.4 Enzyme specific inactivation

Over the last couple of decades a variety of molecules, both naturally occurring as well as synthetic, has been shown to cause specific inactivation of some target enzyme. The outcome of this chemical intervention of the biological system can be beneficial, as in the blockage of microbial growth, or it can as is often the case produce toxic effects.

An understanding at a molecular level of these phenomena in chemical terms can be useful in elucidating how chemical transformations are effected by nature's macromolecular protein catalysts, the enzymes, and in the rational design of drugs with maximal *in vivo* specificity.

At the outset we can divide chemical agents that inhibit specific enzymes into two categories: (i) reversible and (ii) irreversible inhibitors:

(i) Reversible inhibition is characterised by a rapid dissociation of the enzyme-inhibitor complex, and in general requires no chemical transformation by the target system to have their effect.

(ii) Irreversible inhibitors dissociate only very slowly, if at all, from their target enzyme. This slow dissociation is a consequence of the inhibitor binding tightly to the active site of the enzyme usually through covalent bonding.

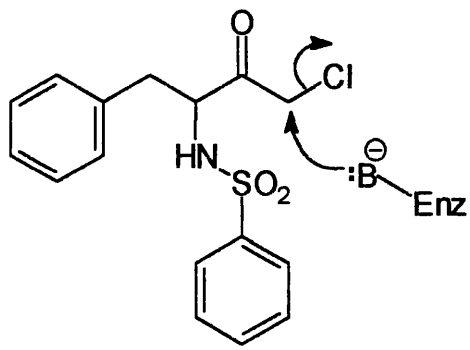
Table 2 - Common enzyme Inactivating functional groups

Types of enzyme inactivating functional groups
Acetylenes
Halogenated substrates
Cyclopropanes
Thionosulfur compounds
Quinones
Allylic compounds
Carbonium ion precursors

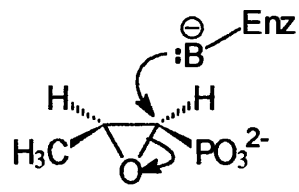
It is irreversible enzyme inhibition with which we will concern ourselves in the remainder of this chapter.

2.5 Irreversible enzyme inhibition

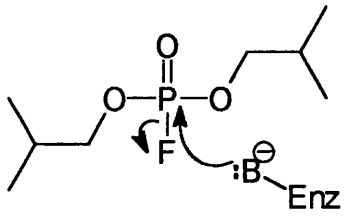
In the broad category of irreversible inhibitors of target enzymes, we can distinguish two groups. The first group are active site directed reagents. These are structural analogues of normal substrates of the target enzyme with one important difference, in that they have a built in reactive group (see table 2) (13). The reactive group could include any of the following:



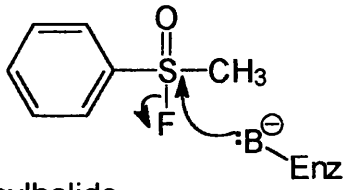
terminal halogenated substrate
e.g. tosyl phenyl chloromethylketone



epoxide
e.g. phosphonomycin



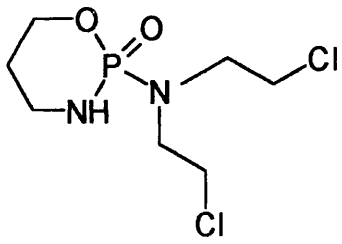
phosphorylhalide
e.g. diisopropylfluorophosphate



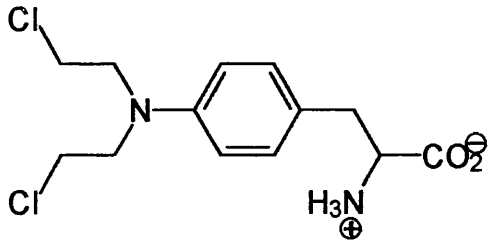
sulfonylhalide
e.g.phenylmethanesulfonylfluoride

These molecules take advantage of binding specificity and the predetermined position of some nucleophilic aminoacid side chain present in the active site of the target enzyme. Once in place the reactive group sets up a displacement reaction for covalent modification and inactivation of the enzyme.

Although useful for active site structural mapping of purified enzymes, these compounds are in general too indiscriminate. The reactive functional groups, are already present in the cellular solution and will tend to react with the first nucleophile they come in contact with. This is often not the target enzyme. Due to the indiscriminate nature of this group of structural analogues they do not have widespread applications except in extreme situations. Some anti-cancer drugs such as cyclophosphamide and phenylalanine mustard fall into this group.



cyclophosphamide



phenylalanine mustard

The second group of enzyme specific and irreversible inactivation reagents has been in medical use for about the last 20 years. They differ from the first group in that the reactive functional group is latent while the molecule is in cellular solution. Only after binding to the target enzyme and subsequent to the enzyme beginning catalysis is the reactive chemical group uncovered. The

inactivators are enzyme specific with the inactivation occurring only in the precise micro-environment of the target enzyme, the active site. If covalent capture is efficient, then only the conscripted enzyme molecule is modified and its catalytic activity destroyed. This class of inactivation has received several terminologies including; mechanism based enzyme inactivation, k_{cat} inhibitors, and suicide substrates (14-19). Although the term suicidal inactivation conveys the role of the enzyme molecule in catalysing its own destruction, it is a misnomer in so far as the enzyme is not doing this deed willingly, but rather by mistake (20).

2.5.1 Mechanism based irreversible enzyme inactivation

In order for a compound to fall into the category of a mechanism-based enzyme inactivator it must meet certain chemical and kinetic criteria:

i) As with any substrate where physical binding to the enzyme active site precedes catalysis, the inactivator should follow the simple minimal scheme, where a binding equilibrium following first order kinetics leads to inactivation.

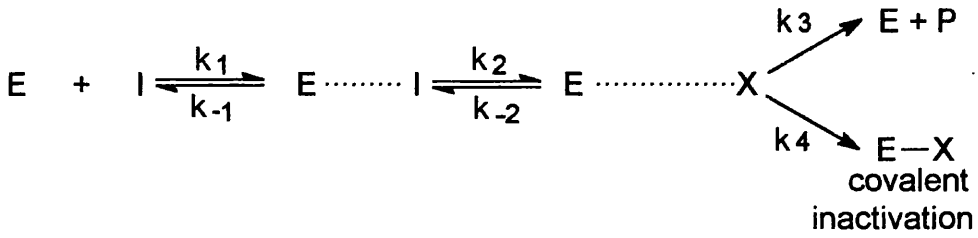
ii) If the covalent modification that leads to enzyme activity destruction is a mechanism-based process that derivatises a key aminoacid residue of the enzyme, the chemical stoichiometry of modification should be a 1:1 adduct / enzyme ratio.

In the case of some multimeric enzymes, substoichiometric labelling can cause complete activity loss. Thus when the bacterial enzyme methionine-γ-lyase is inactivated by propargylglycine, 2 labels per tetramer block all activity. Presumably the two modified sub units show altered conformations that are transmitted by inter-subunit contacts to the unmodified subunits (21). Higher stoichiometry values probably reflect non-specific alkylation processes (22).

iii) A final criterion which is both useful mechanistically in identifying the nature of the inactivating species and also in the evaluation of any *in vivo*

therapeutic potential is the partition ratio, the simple k_3 / k_4. This represents the number of times a suicide substrate is processed by the enzyme to produce a product (P) without harming the enzyme, for every inactivation that occurs.

The mechanism-based class of activators requires the enzyme go at least part way through its normal chemical catalytic cycle before the latent functionality is uncovered. Therefore in designing a drug we would require to know how often the catalytic cycle is completed compared to how often it is re-routed into covalent derivatisation of the enzyme. Maximal specificity will be represented by complete derivatisation of the enzyme.



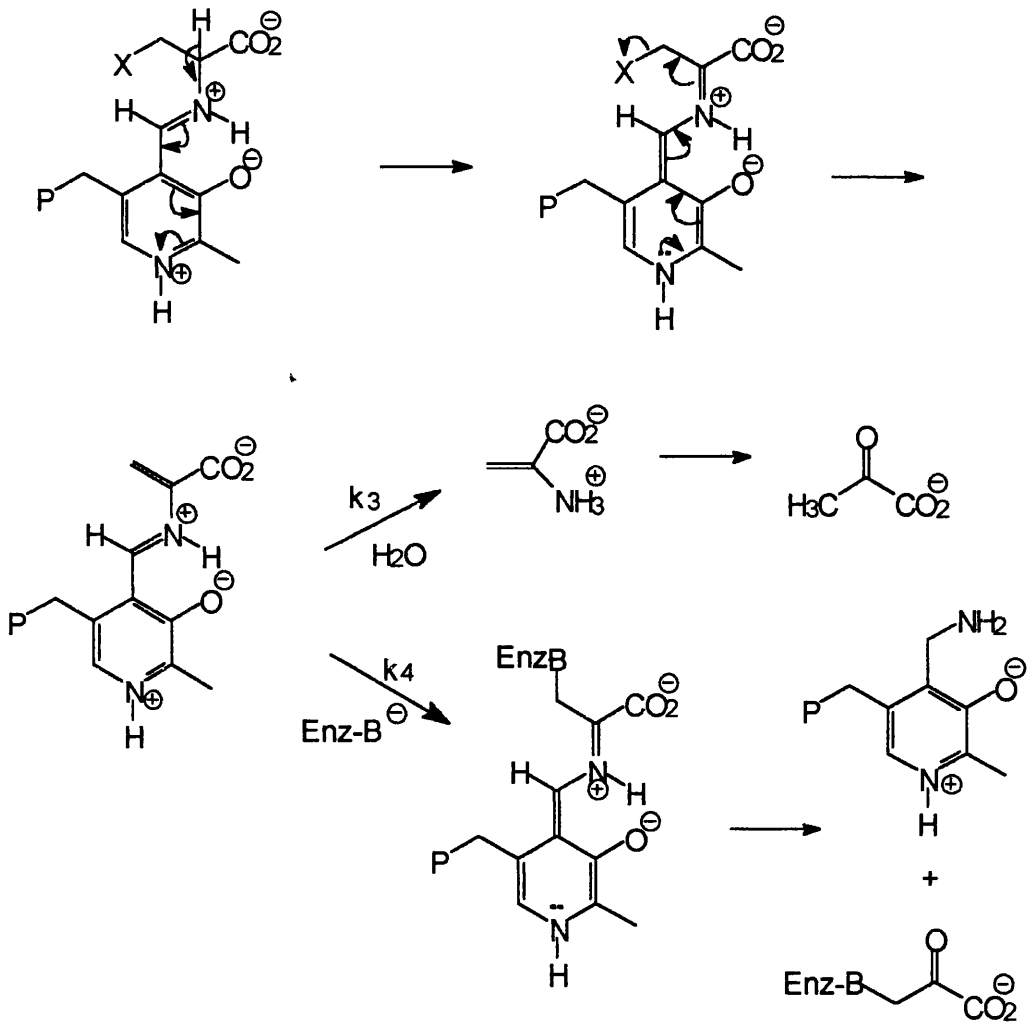
When route k_3 is followed molecules of P produced and released are unlike the latent groups in the substrate and are fully uncovered and reactive. These reactive product molecules can then diffuse to other cellular components and alkylate or acylate them. This on a microscopic scale is a loss of specificity and on a macroscopic scale would be a source of side effects or toxicity.

In the drug design approach of mechanism based enzyme inactivation, the two classes of functional groups with which we will concern ourselves are halogenated substrates and allylic compounds.

2.6 Halogenated substrates

In recent years a large number of compounds have been found to function as suicide substrates through loss of a halide or other good leaving group. The mechanism of enzyme inactivation involves carbanion formation, at a site

adjacent to the leaving group, generating a conjugated allylic system. This conjugated system then undergoes Michael addition to release the leaving group and yield the alkylated and deactivated enzyme (23). Since pyridoxal dependent enzymes specialise in generating α- and β-carbanion equivalents during catalysis, almost all the target enzymes in this group are pyridoxal dependent enzymes. Since aminoacids are substrates of pyridoxal phosphate dependent enzymes, β-halo-α-amino acids can act as suicide substrates for this class of enzymes.



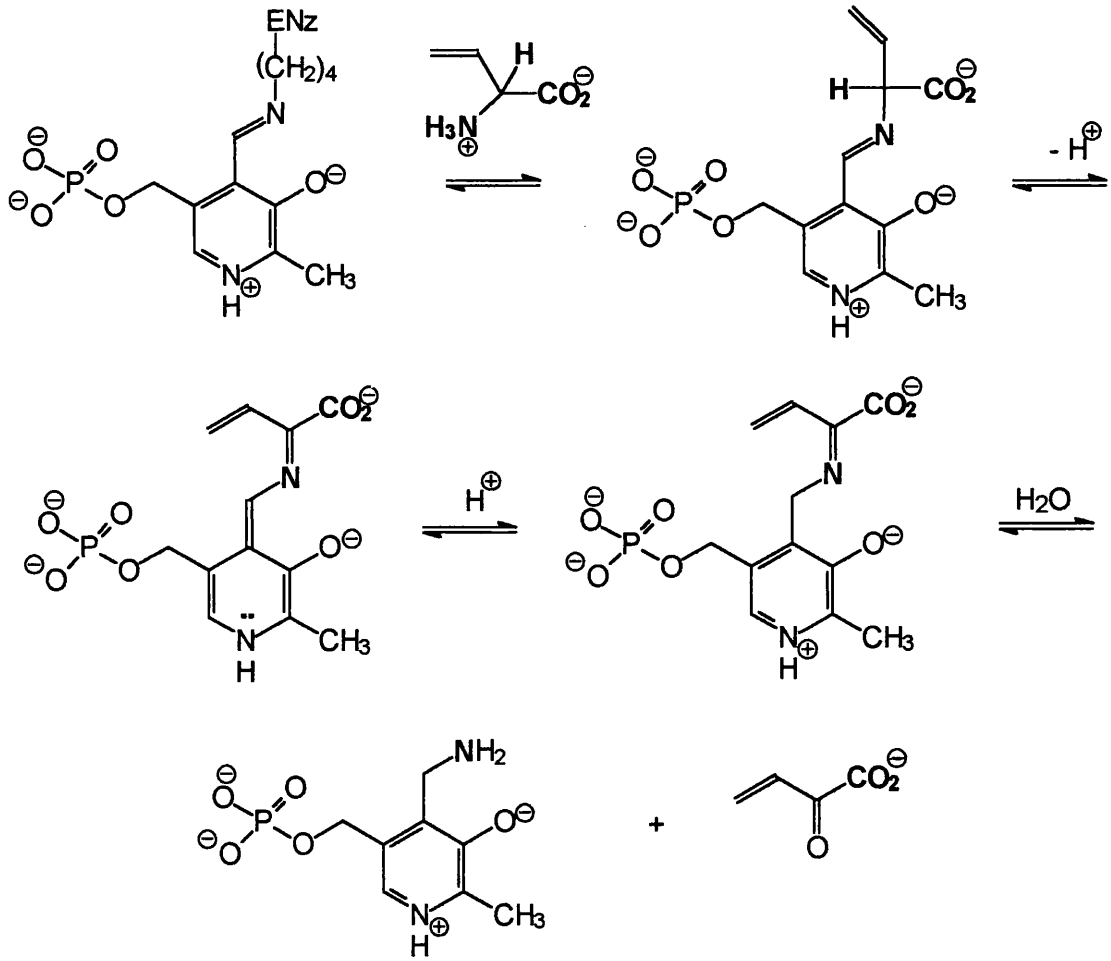
P= $PO_4^{2\ominus}$

Mechanism of pyridoxal phosphate dependent enzyme inactivation

e.g. Replacing the leaving group X with F (fluorine) in the above scheme demonstrates the method by which β-fluoroalanine inactivates the bacterial enzyme alanine racemase on a molecular level (24, 25).

2.7 Allylic compounds

Although some enzymes are able to process allylic functionalities most cannot. By introducing a reactive allylic bond in the active site of an enzyme which does not routinely encounter any it is possible to trigger mechanism based alkylative inactivation of the enzyme. To demonstrate allylic bond deactivation we can look to the γ-substituted α-amino acid vinylglycine. In the transamination pathway when vinylglycine is transaminated by a pyridoxal phosphate dependent transaminase, the bound product can then either be released or captured in an inactivating Michael attack as shown (26, 27).



transamination of vinylglycine

Chapter 3

Fluorine in Medicine in the Twentieth Century

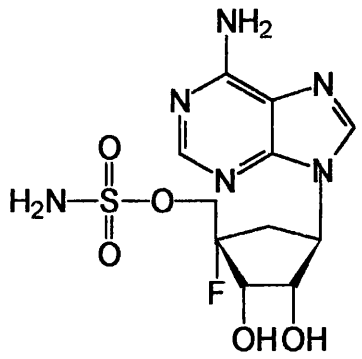
The unique properties of fluorine make it a very useful addition to the medicinal chemist's toolbox. The importance of fluorine in successful drug design and in the pharmaceutical industry in general is increasingly reflected in the growing proportion of new drugs containing fluorine.

3.1 Fluoroorganic chemistry - a brief history

Fluoroorganic chemistry is almost entirely a man-made subject and the fluorocarbon branch of chemistry has jokingly been described as 'unnatural product chemistry'.

Only 10 naturally occurring fluoro metabolites have been discovered so far and they are all monofluorides. Monofluoroacetic acid was the first to be discovered in 1944 and isolated from the South African plant *Dichapetalum cymosum* (28, 29, 30). Monofluoroacetic acid is now known to occur in more

than 30 other plant species and is produced by the bacterium, *Streptomyces cattleya*. Another naturally occurring fluorine containing product is the antibiotic fluoro sugar nucleocidin, produced by the soil bacterium *Actinomycete* of the genus *Streptomyces*.



nucleocidin (natural product used as an antibiotic)

Bench chemists have easily outstripped nature (31), having so far reported in the region of 7 X 10^5 compounds containing one or more C–F bond. The first and simplest fluoroorganic compound, methyl fluoride, was synthesised in 1835 by Dumas and Péligot (32). Dumas and Péligot described the reaction as a displacement of the sulphate group by fluorine, a reaction of the type now described as an S_N2 displacement.

$$2KF + (CH_3O)_2SO_2 \rightarrow 2CH_3F + K_2SO_4$$

S_N2 displacement reactions using fluoride are now amongst the most widely used methods of synthesising aliphatic C–F bonds.

The next major step forward in organo fluorine chemistry did not come until the early 1880's, when Palernò and Oleveri synthesised monofluorobenzene utilising the then emerging arenediazonium mediated methodology.

$$ArN_2^+ + F^- \rightarrow ArF + N_2$$

In 1898 the Belgian chemist Swarts reported the conversion of phenyl trichloromethane ($C_6H_5CCl_3$) to phenyl trifluoromethane ($C_6H_5CF_3$) now a fluoro aromatic building block of great commercial value (32). At this time Swarts was already considering ways of incorporating fluorine into aliphatic hydrocarbons. By the turn of the century and via aliphatic halogen exchange reactions involving metal fluorides and in particular antimony fluorides, e.g. SbF_3 now known as Swarts reaction reagents, Swarts was able to synthesis the first fluorinated aliphatic hydrocarbons. Swarts' discoveries provided the impetus in the 1930's for the production of chlorofluorocarbon refrigerants (brand name *Freon*), marking the first commercial application of organo fluorine chemistry. This was quickly followed by the discovery by Plunkett and Rebok of the polymer poly(tetrafluoroethylene) which was produced for use in World War II but not fully commercialised until 1950 under Dupont's *Teflon* brand and ICI's *Fluon* brand in 1956 (32). It was also around this time that the first real progress in biochemical applications of fluorine were made.

3.1.1 Biochemical applications of fluorine

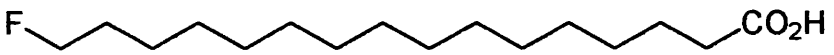
In the mid 1880's Peter Griess prepared the first fluorine analogue of a natural product, fluorohippuric acid $C_6H_4FCONHCH_2CO_2H$, but it was not until 1948 during World War II that the first real progress in biochemical applications was made. During World War II the prominent biochemist Sir Rudolph Peters, through his pioneering work on fluoroorganic compounds, demonstrated the toxic effects of monofluoroacetic acid. He showed that monofluoroacetic acid has no effect on acetate metabolism, but induces the toxic accumulation of acetic acid in the kidneys and liver of a guinea pig (33). This discovery led to the concept of 'lethal synthesis'. In this case monofluoroacetic acid is converted metabolically to a 2-fluorocitrate diastereoisomer (2R, 3R) which is thought to exert its toxic effect through inhibiting the enzyme mediated isomerisation of citrate to iso-citrate in the mitochondria (34).

$CH_2FCO_2^-Na^+$ $\qquad$ $LD_{50}^{rat} = 0.2mg/Kg$

Note: The lethal dosage for a 70kg man is *ca* 400mgs.

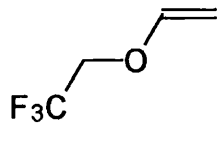
Therefore in any drug design approach careful attention must always be given to metabolic pathways to ensure monofluoroacetic acid is never a byproduct. A well known and striking example of fluorotoxicity is found in ω-fluoroalkylcarboxylic acids. $H_2CF(CH_2)_XCO_2H$. When X is an even number; X = 2,4,6, etc. the carboxylic acid is toxic. When X is an odd number; X = 1,3,5, etc. the carboxylic acid is non toxic. This example has been hailed as a beautiful verification of the theory of β-oxidation of fatty acids. Because they are biosynthesised from the 2 carbon acetyl groups in acetyl coenzyme A, almost all naturally occurring fatty acids have an even number of carbon atoms.

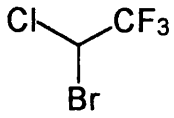
Example:

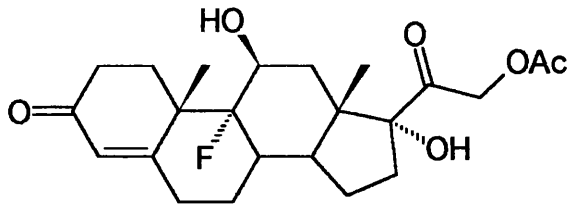


ω-monofluoropalmitic acid is toxic

From the late 1950's to the present day represents a period of major advancement in the history of medicine. Fluorine containing medicines, frequently made available by the use of new reagents and improved handling techniques for the introduction of fluorine, have contributed greatly to these advances. Notable historical events during this time include the first commercial fluorinated inhalation anaesthetics Fluoroxene (1953) and halothane (1956) (35, 36) and the pioneering work of Fried and Sabo in 1954 on the preparation of 9α-fluorohydrocortisone acetate (37). Fried and Sabo's work led to the first significant reporting of a useful, not harmful, modification of biological activity through selective fluorination. In tests on rat liver glycogen Fried and Sabo found that 9α-fluorohydrocortisone acetate possesses 11 times the activity of hydrocortisone acetate.



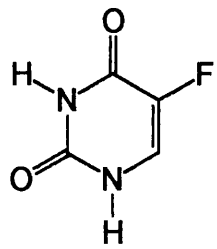




fluorooxene halothane 9,α-fluorohydrocortisone

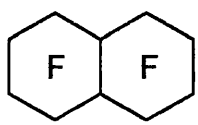
historical inhalation anaesthetics first fluorinated steroid analogue

C. Heidelberger and coworkers in 1956, through their investigations on the fluorinated pyrimidine 5-fluorouracil made significant advances in the treatment of cancer (38). Since then a great deal of the medicinal chemists' time has been spent synthesising and screening fluorinated pyrimidines for tumour inhibiting anticancer and antiviral activity.

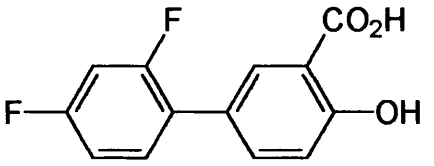


5-fluorouracil (1956), nucleic acid antagonist

Over the past 40 years the wide range of applications of fluorine in medicine has been phenomenal and for perspective a cross section of examples is given below:



perfluorodecalin (1970), blood substitute



diflunisal (1977), salicylic acid analogue

A nonsteroidal anti-inflammatory drug used in the treatment of osteoarthritis. It is quickly absorbed into the body and is much more effective than aspirin.

$CF_3(CF_2)_7Br$

perflubron (1993), (*Imagent* - perflubron emulsion), oxygen transport / sonography contrast agent

$[-CH_2-CF_2-]_n$

polyvinylidene fluoride (synthesised 1960's)

With its good thermal properties, mechanical strength and chemical inertness polyvinylidene fluoride can be processed into precision parts for orthopaedic applications (1990's).

3.2 Fluorine the enabler

So what advantage does fluorine offer? The incorporation of fluorine into a molecule with the aim of improving therapeutic efficiency is based on several key considerations (32):

- Fluorine is the second smallest substituent after hydrogen and closely mimics hydrogen with respect to steric requirements at the enzyme receptor.

van der Waals radius [Å]

$CH_3 = 2.1$ $CF_3 = 2.5$ $CCl_3 = 3.5$ $CCH_3 = 3.5$

- Fluorine is the most electronegative element and it frequently alters the electron density, basicity and acidity of neighbouring groups and thereby their chemical reactivity.

Electronegativity (according to Pauling)

$H = 2.1$ $C = 2.5$ $F = 4.0$

- The strength of the C–F bond exceeds that of the C–H bond. This difference often results in increased thermal and oxidative stability of the modified substrate. This property has been exploited in attempts to protract the action of a drug by blocking the sites involved in oxidative degradation.

Bond energies [kJmol^{-1}]

$C–H = 416$ $C–F = 485$ $C–Cl = 328$

- The replacement of hydrogen by fluorine increases lipid solubility. The CF_3 group is amongst the most lipophilic of all the substituents. This property may contribute to the absorption, transportation and delivery of the modified substrate to its biological target. The effective concentration of the drug in the target tissue can thus be increased by improving the ability of the molecule to penetrate the lipid bilayers.

Lipophilicity [π]

$CH_3 = 0.50$ $C_2H_5 = 1.00$ $CF_3 = 1.07$

- Only a few higher plants and bacteria are able to metabolise inorganic fluoride, and naturally occurring organofluorine compounds are extremely rare (39-43). Therefore metabolic breakdown or transformations of the modified substrate, as a consequence of fluorine incorporation, is highly unlikely.

3.3 Fluorine containing anti-viral and antibacterial agents

In the past couple of decades medicinal chemists have been rigorously exploring the use of fluorine as a replacement for hydrogen at sites which are subject to metabolism, with a particular focus on viral and bacterial metabolic pathways.

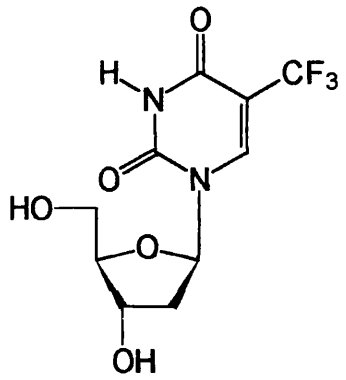
With the discovery of AIDS (and the HIV virus) in the 1970's it has in the years since become increasingly apparent that AIDS and other immuno-compromised patients are particularly vulnerable to viral and fungal infections. In many cases if left untreated these infections can eventually lead to the death of the immuno-compromised individual.

In the field of anti-viral and anti-fungal agents there exist many examples where a methyl group has been replaced by either fluorine or trifluoromethyl with retention of biological activity.

3.3.1 Fluorine containing anti-viral agents

More than three decades of focused anti-viral research (spanning the 1960s - 1990s) have yielded only a handful of drugs approved by the FDA (Food and Drug regulatory Authority) for the treatment of human viral diseases.

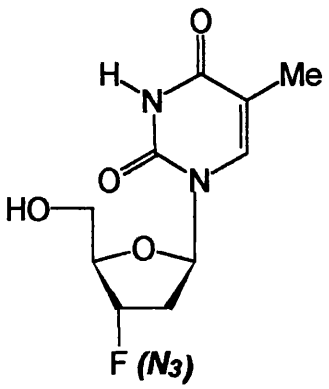
Trifluorothymidine was the first fluorine containing anti-viral drug to be marketed. Trifluorothymidine was designed as a thymidine mimetic and was first synthesised in 1962 as part of a programme directed towards the discovery of anti cancer agents (44). However its biological activity is mediated through phosphorylated metabolites and this limits its clinical application to the topical treatment of the HSV (Herpes Simplex Virus) virus.



trifluorothymidine

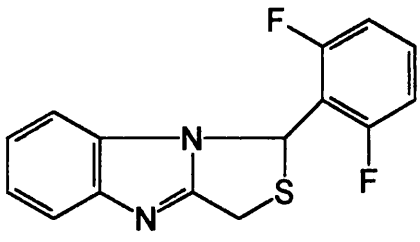
Designed as an analogue of thymidine, 3'α-fluoro thymidine was reported in 1988 as having wide ranging anti-viral activity (45). It is more potent than AZT against HIV and was progressed to clinical trials in AIDS patients. Unfortunately development of this drug was terminated through its failure to produce efficacy at doses with acceptable side effect profiles (46).

Among the class of randomly screened compounds TBZ has been reported as possessing anti HIV activity, attributed to its ability to inhibit reverse transcriptase (47). However as far as I am aware this compound was never progressed to full scale clinical trials.



3'α-fluorothymidine

(AZT)

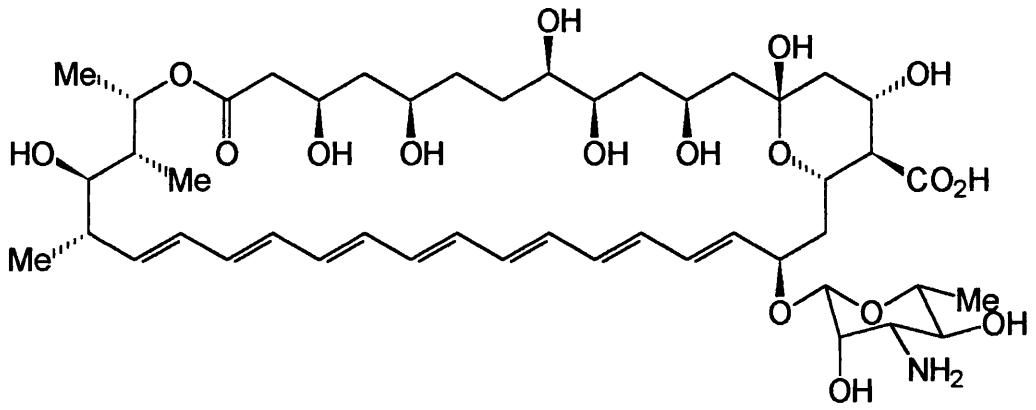


TBZ

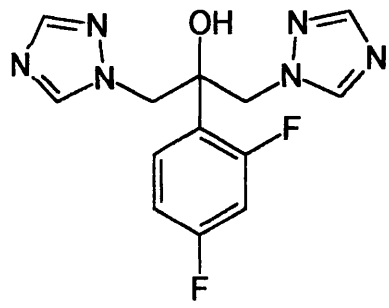
In the fight against AIDS the search for a more effective (even curative) anti-viral agent with minimal to no side effects continues.

3.3.2 Fluorine containing anti-fungal agents

Until recently the choice of anti-fungal therapies was limited (48), with amphotericin B being the only drug of choice in the treatment of systemic fungal infections. However use of amphotericin B is accompanied by several drawbacks including the need to administer it via slow intravenous infusion over several hours and the risk of adverse side effects of fever, nausea and renal toxicity. The discovery of the fluorine containing drug fluconazole in 1993 by the pharmaceutical company Pfizer, marked a major step forward in the treatment of anti-fungal infections (49). Fluconazole can be given orally or intravenously and its excellent safety profile means that doses of up to 2g per day are well tolerated by patients with few if any side effects. Today fluconazole is the global market leader in the treatment of anti-fungal infections.



amphotericin B, anti-fungal agent



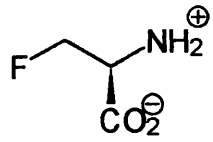
fluconazole (*Diflucan* - brand name), anti-fungal agent

3.3.3 Fluorine containing amino acids

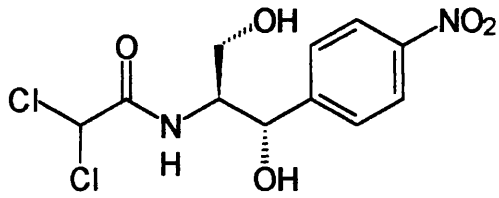
Even if we were to disregard the unique importance of amino acids in the initiation of life on earth, compounds of this class possess a sufficient number of biological functions to be of fundamental necessity to all living organisms. The synthesis of amino acids and their analogues must therefore be considered to occupy a position of great importance in modern bioorganic chemistry and in the creation of new medicines.

The vigorous development of organofluorine chemistry since the 1940's, together with recent advances in biochemical understanding have created a wave of interest in the research of fluorine containing amino acids. The interest in these compounds is in many respects due to the fact that most of them are proving to be highly selective inhibitors of 5' pyridoxal phosphate dependent enzymes.

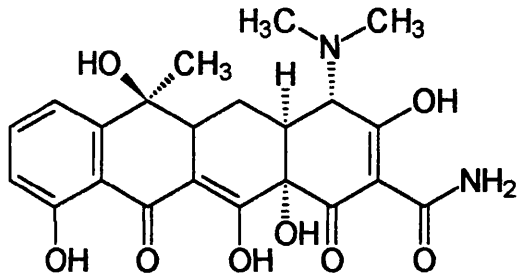
The first successful fluorination of an amino acid as part of an anti-fungal drug design approach came in 1970 with the discovery of the novel anti-fungal β-fluoro-D-alanine. This structurally simple molecule was found to be of equal activity to tetracycline and chloramphenicol with respect to *in vivo* anti-bacterial activity in mice. Microbiologists were later able to prove that in agreement with design ideas, anti-bacterial β-fluoro-D-alanine interferes with the biosynthesis of D-alanine via enzymatic deactivation of alanine racemase (24, 25).



β-fluoro-D-alanine



chloramphenicol



tetracycline

anti-bacterial agents

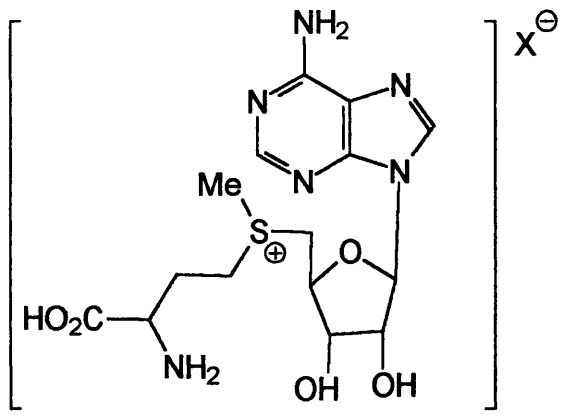
Chapter 4

Methionine and Methionine-γ-lyase as Agents for Drug Design

Following a rational drug design (RDD) approach, it was our aim to synthesise potential anti-metabolites for the therapeutic treatment of infections arising from anaerobic micro-organisms and helminths. As discussed in chapter 1 the first step of the RDD was to identify a metabolite of key significance to a biological process.

4.1 Key metabolite - L-methionine

There is a great diversity of organic sulfur compounds which are of fundamental importance biologically. The sulfur containing amino acid L-methionine plays diverse biochemical and physiological roles not only as a constituent of peptides and proteins but also as a precursor in the biosynthesis of 5-adenosylmethionine. 5-Adenosylmethionine is involved in many important biological processes and acts as a methylating agent in the methylation of DNA and proteins. 5-Adenosylmethionine also plays a role in the biosynthesis of polyamines, essential elements in cellular growth (50, 51).

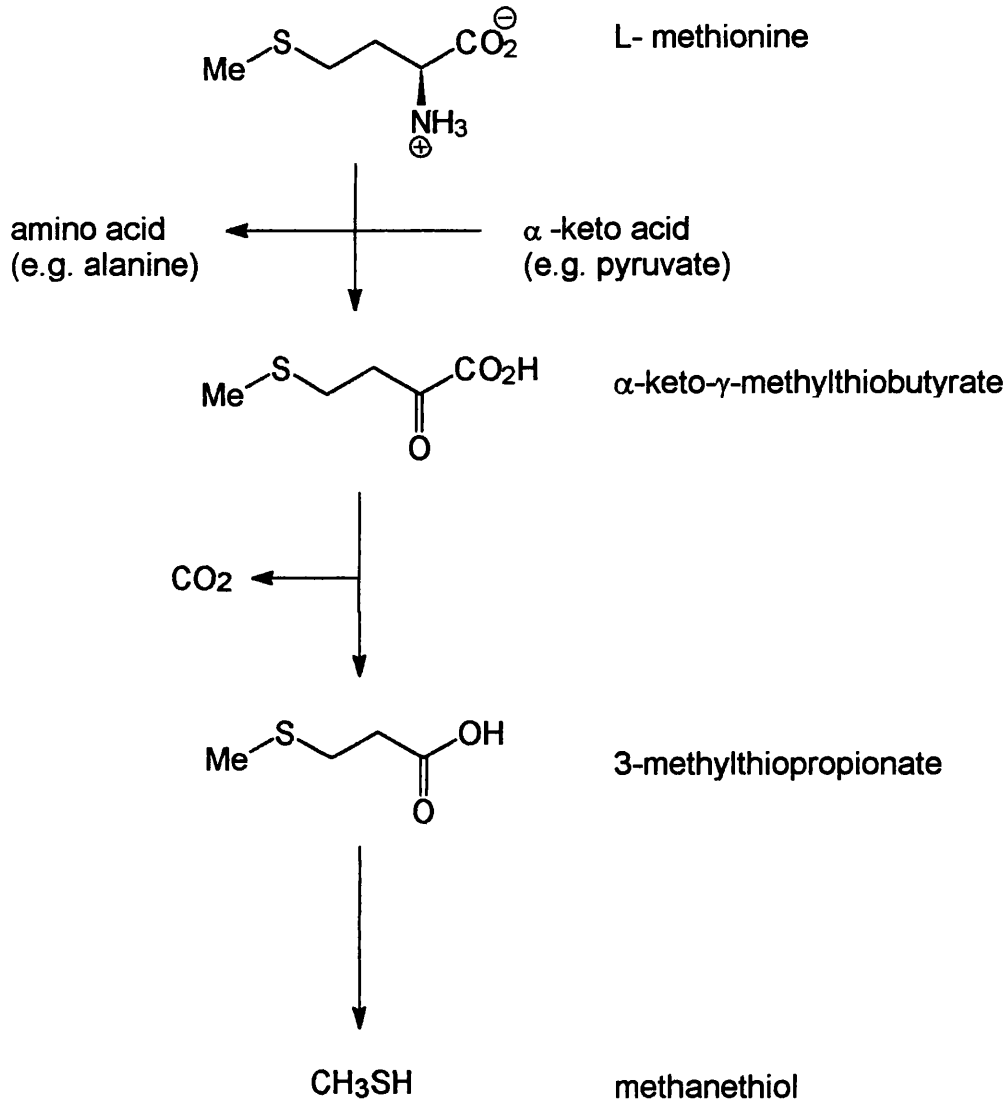


5-adenosylmethionine

Thus preparation of analogues of methionine as potential anti-metabolites holds promise for the treatment of disease because of the metabolic significance of the parent compound.

4.2 Metabolic pathway of L-methionine in mammals

In mammalian cells L-methionine catabolism has been identified as following the transaminative pathway (50, 52, 53).



transaminative pathway showing the catabolism of L-methionine in mammalian cells

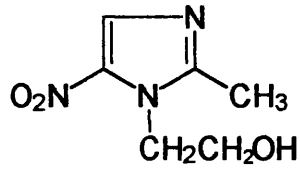
A similar mechanism has been proposed for the catabolism of methionine in **aerobic** bacteria and fungi (54, 55).

4.3 Trichomonas vaginalis and trichomoniasis

Trichomonads, including *Trichomonas vaginalis and Trichomonas foetus* which are parasites of the urogenital tracts of humans and cattle respectively, are anaerobic flagellate protozoa. Cysteine and related thiol compounds are

thought to play important roles in countering oxygen toxicity and maintaining the cell integrity of these anaerobic flagellate protozoa.

Abortion and sterility are symptomatic of cattle infected with *Trichomonas foetus*, while *Trichomonas vaginalis* is responsible in humans for the sexually transmitted disease trichomoniasis. Although some colonies of *Trichomonas vaginalis* are assymptomatic most are not, producing in men a burning sensation during urination and in women an itching or burning sensation of the vagina accompanied by a foul smelling discharge. It is estimated that up to 20% of the human population may be or may have been infected with this disease. In the past *trichomoniasis* was usually treated using 5-nitroimidazoles such as metronidazole (commercially available as *Flagyl, Metrogel, Metrolyl, Vaginyl* amongst others trade names) and nimorazole. However these agents can give rise to resistant strains of the parasite and are suspected of having mutagenic effects. Thus demonstrating the need for new pro-drugs of greater specificity to the disease process.



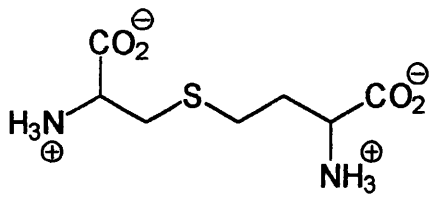
metronidazole, (trade name *Flagyl,* etc.)

It is *Trichomonas vaginalis* upon which our drug design discussion will centre on in the remainder of this chapter. *Trichomonas vaginalis* is known to contain operative amounts of the enzyme methionine-γ-lyase.

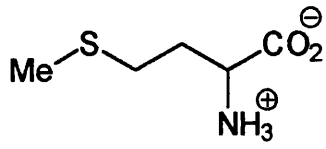
4.4 Methionine-γ-lyase

The enzyme methionine-γ-lyase catalyses the breakdown of L-methionine to α-ketobutyrate, ammonia and methanethiol in some bacteria, but is apparently absent from mammals. The D-enantiomer of methionine is not a substrate for

the enzyme, nor does it inhibit competitively the enzyme's activity. This indicates that the L-configuration at the α-carbon is necessary for binding of the substrate to the active site. The purified methionine-γ-lyase of *T. vaginalis* has no activity towards the substrate cystathionine and is therefore clearly distinct from the mammalian enzyme γ-cystathionase (56, 57). (Cystathionine purified from the rat liver can participates in enzyme catalysed α- and γ-elimination reactions and is a known substrate of the mammalian pyridoxal phosphate dependent enzyme γ-cystathionase).



cystathionine

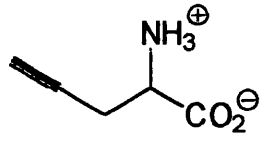


methionine

4.5 L-methionine-γ-lyase as a target enzyme for drug design

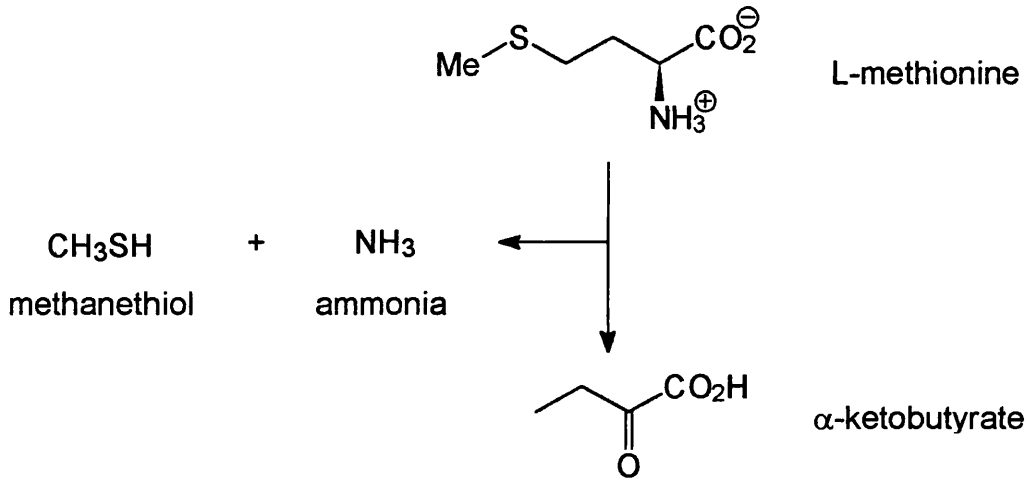
The enzyme L-methionine-γ-lyase of *T. vaginalis* is similar in many ways to bacterial L-methionine-γ-lyase, both enzymes catalyse γ-eliminations (e.g. with methionine or ethionine) and β-eliminations (e.g. with S-methyl-L-cysteine) reactions (56, 58).

Inhibition of L-methionine-γ-lyase in *T. vaginalis* by known inhibitors of 5-pyridoxal phosphate dependent enzymes such as D,L-propargylglycine indicates that the parasite enzyme is a pyridoxal phosphate dependant enzyme.



propargylglycine

When cell free extracts of *T. vaginalis* were presented with α-keto-γ-methiolbutyrate, an important intermediate in mammalian degradation of methionine, a low rate of catabolism was observed. This suggests that α-keto-γ-methiolbutyrate is not an intermediate in the main route of methionine catabolism by *T. vaginalis*. In studies it was demonstrated that *T. vaginalis* catabolises L-methionine by simultaneous deamination and dethiomethylation to give α-ketobutyrate, ammonia and methanethiol (56, 59, 60).



L-methionine catabolism in *Trichomonas vaginalis*

When compared with other trichomonads it is surprising to find that L-methionine-γ-lyase is present at relatively high concentration in only *T. vaginalis*. A major difference between trichomonal and bacterial L-methionine-γ-lyase is the lack of detectable NH_3 with the trichomonal enzyme. Both these findings suggests the enzyme performs a function peculiar to *T. vaginalis* and that the differences could be a source of increased specificity for the disease process and possible commercial exploitation.

It has been suggested that the production of methanethiol in *T. vaginalis* may be linked to the interactions of the parasite with its host and other micro-organisms present at the site of infection, the most important site being the human vagina. The production of methanethiol affects mammalian cells and

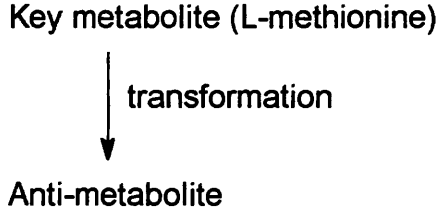
other vaginal flora micro-organisms and is the cause of the foul smelling odour often a characteristic symptom of the disease trichomoniasis. Interestingly it has been documented that some higher plants produce volatile thiols as repellants against predators and parasites (52, 61-66).

L-methionine-γ-lyase may play a part in the survival of the parasite within the host and the host bacterial environment and also in its pathogenic role. Support for this suggestion comes from the observation that the enzyme does not appear to be essential to the activity of *T. vaginalis* in axenic culture (a culture medium in which only one type of micro-organism is growing - used to determine basic growth requirements in microbiology).

Since L-methionine catabolism in *T. vaginalis* differs from that found in mammals and other vaginal flora bacteria, the enzyme L-methionine-γ-lyase presents itself as a target for therapeutic attack. If the suggested role of the enzyme is correct, then specific inhibitors of L-methionine-γ-lyase could significantly reduce or even cure the pathogenicity of intravaginal trichomonal infections.

4.6 Summary of the rational drug design process so far

Target Disease Process	⟶	trichomoniasis
Key Metabolic Process	⟶	L-methionine catabolism in *Trichomonas vaginalis*
Specificity	⟶	target enzyme - methionine-γ-lyase
Key Metabolite	⟶	L-methionine



Summary of identified key components in the rational drug design process

Having now chosen, via the drug design approach, the target enzyme and key metabolite the next step was to transform the selected metabolite into an anti-metabolite. The basic notion of forming an anti-metabolite is to modify the metabolite maximally with respect to chemical character, but minimally with respect to distortion of geometry, now termed isogeometric modification. The role of L-methionine as a methyl donor suggests that substitution of another group such as fluorine or trifluoromethyl for the essential methyl might prove to be of particular value.

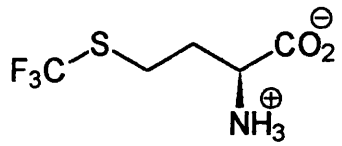
The chapter that follows will discuss ways of synthesising methionine anti-metabolites by incorporating fluorine into methionine and related compounds with a particular emphasis on the thiomethyl moiety.

Chapter 5

Synthesis of Fluorinated Analogues of L-methionine and Related Compounds

At the outset of this research programme and in collaboration with Professor G.H. Coombs' research group at the Department of Parasitology, University of Glasgow, it was agreed that a suitable starting point for this project would be the efficient preparation of L-trifluoromethionine.

Professor G.H. Coombs' research group was and still is developing methods to elucidate the metabolic pathways of trichomonads and how they interact with mammalian cells, focusing in particular on the mechanism of action and inhibition of the enzyme methionine -γ-lyase in *Trichomonas vaginalis*.



L-trifluoromethionine

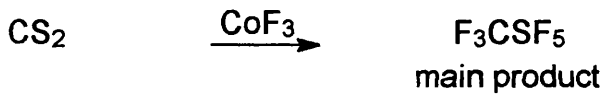
5.1 Historical developments in fluorine chemistry leading to the preparation of the first fluorinated analogue of methionine

The synthesis of fluorinated sulfur containing amino acids has been led mainly by developments in fluorine chemistry and the advent of more user friendly preparative techniques and reagents for the introduction of fluorine.

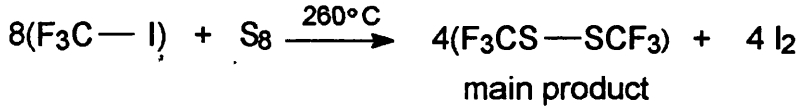
Synthesis of organic molecules containing fluorine can be achieved in one of two ways. One can either i) introduce fluorine directly using a fluorinating agent such as an alkali metal fluoride (this method often requires special equipment and handling expertise), or ii) one can follow an indirect route and use fluorinated building blocks (reagents which already have the desired C-F bond(s)). This second option is usually preferred by non fluorine specialists and has been the most popular method of choice in the reported preparation of fluorinated analogues of methionine.

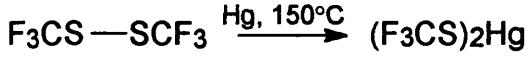
5.1.1 Early perfluoroalkyl derivatives of sulfur and sulfur containing compounds

It was not until the 1950's that the first reported perfluoroalkyl derivative of sulfur was made by Silvey and Cady (67). In their report they describe the fluorination of carbon disulfide by cobalt trifluoride to yield trifluoromethyl sulfur pentafluoride as the main product.



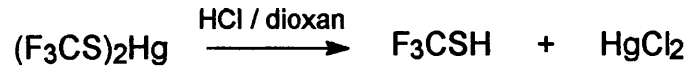
A couple of years later in 1952, G.A.R Brandt and co-workers (68, 69) showed that trifluoromethyl iodide and sulfur could react in a stainless steel autoclave at 260°C to give bis(trifluoromethyl) disulfide $C_2S_2F_6$ as the main product. One year later, in 1953, R.N. Hazeldine and J.M. Kidd published a report (70) describing the synthesis of mercuric trifluoromethyl mercaptide from the reaction of bis(trifluoromethyl) disulfide with mercury.

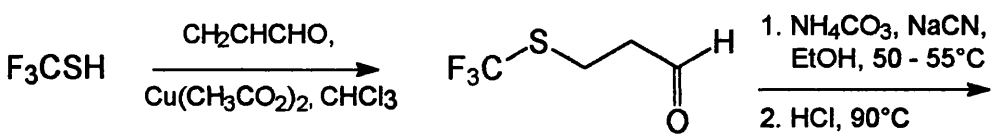


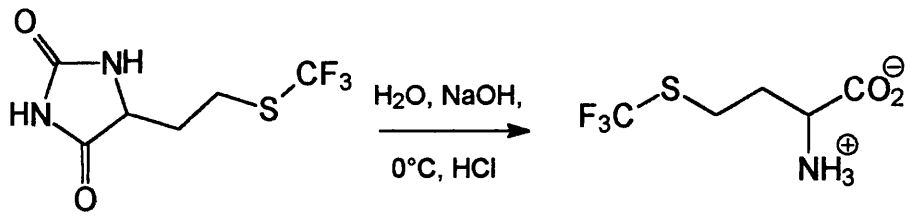


5.1.2 The first synthesis of D,L-trifluoromethionine

In 1957, taking their lead from Hazeldine and Kidd's work on the synthesis of mercuric trifluoromethyl mercaptide, R.L. Dannley and R.G. Taborsky were the first to synthesise D,L-trifluoromethionine (71). Their synthesis involved first converting mercuric trifluoromethyl mercaptide to the mercaptan, then reaction of the mercaptan with acrolein in the presence of cupric acetate to yield trifluoromethyl mercapto propionaldehyde. Trifluoromethyl mercapto propionaldehyde was then easily converted to the D,L-trifluoromethionine in 3% overall yield.







D,L-5-(β-trifluoromethylmercaptoethyl)hydantoin D,L-trifluoromethionine

At this time neither Dannley and Taborsky or any other research group reported any attempt to improve the yield or to test trifluoromethionine for biological activity. It was not until the advent of radical ion trifluoromethylation and its applications in the perfluoroalkylation of aliphatic hydrocarbons that the synthesis of fluorinated analogues of methionine was revisted.

5.2 Radical ion trifluoromethylation

In the early 1970s Bunnett and co-workers demonstrated that unactivated aryl halides readily enter into reactions with nucleophilic agents in liquid ammonia after initiation by potassium or U.V. irradiation (72-74). For these reactions they proposed a nucleophilic $S_{RN}1$ mechanism involving the generation of the radical anion of the initial molecule, its conversion into a radical and subsequent reaction of the radical with the nucleophilic reagent.

V.N. Boiko and associates in 1977 (75), building on the earlier work of Bunnett and coworkers, proposed that perfluoroalkyliodides would also be capable of reacting with nucleophilic agents in an analogous type of reaction. This was in spite of the opposite polarisation of the C–I bond compared with alkyl and aryl iodides.

In terms of chemical characteristics perfluoroalkyl iodides differ greatly from their hydrocarbon analogues. This is explained by the opposite polarisation of the trifluoromethyliodide $CF_3^{\delta-}–I^{\delta+}$ to that of alkyl halides $CH_3^{\delta+}–I^{\delta-}$, which gives rise to greater electronegativity in the CF_3 compared with iodine (76, 77) As a

result of this difference in electronegativity, perfluoroalkyl iodides do not possess alkylating characteristics.

Boiko and associates found that trifluoroiodomethane alkylates aliphatic, aromatic and heterocyclic thiols during U.V. irradiation in a pyrex flask. In addition their paper reports that trifluoromethylation of thiols can be realised not only in liquid ammonia but also in organic solvents such as methanol, acetone or acetonitrile in the presence of alkaline agents such as sodium hydroxide or sodium methoxide, although better results are obtained using sodium methoxide. This is probably a consequence of water formation by the action of sodium hydroxide on thiols.

The mechanism of trifluoromethylation of thiols used by Boiko is evidently consistent with the $S_{RN}1$ type mechanism proposed by Bunnett and coworkers for the action of arylhalides on thiophenols.

$S_{RN}1$ mechanism

$$RS^{\ominus} + CF_3I \longrightarrow RS^{\bullet} + (CF_3I)^{\bullet -}$$

$$(CF_3I)^{\bullet -} \longrightarrow CF_3^{\bullet} + I^{\ominus}$$

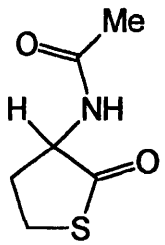
$$RS^{\ominus} + CF_3^{\bullet} \longrightarrow (RSCF_3)^{\bullet -}$$

$$(RSCF_3)^{\bullet -} + CF_3I \longrightarrow RSCF_3 + (CF_3I)^{\bullet -}$$

5.3 Synthesis of di and trifluoromethionine

In 1989, and following an upsurge of interest in the scientific community in general towards fluorinated amino acids and their application in medicine, M.E. Houston Jr and J.F. Honek, set about investigating how they could more efficiently synthesise fluorinated analogues of L-methionine. In their communication of 1989 (78) they describe the synthesis of di and trifluoro analogues of L-methionine.

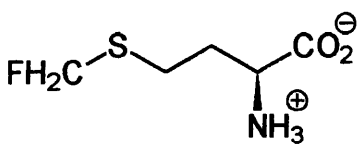
Houston and Honek were able to synthesise (i) L-difluoromethionine (previously unknown) and (ii) L-trifluoromethionine (previously prepared in 3% overall yield by Dannley and Taborsky) from N-acetyl homocysteine thiolactone.(78), affording overall yields of 24% and 26% for L-difluoromethionine and L-trifluoromethionine respectively. Although Houston and Honek had proposed to conduct biological studies on these compounds, to our knowledge no results were ever published.



N-acetylhomocysteinethiolactone

5.4 Synthesis of monofluoromethionine

In addition to synthesising di and trifluorinated analogues of L-methionine, Houston and Honek in the same paper of 1989 (78) reported the synthesis of L-monofluoromethionine. Their synthesis involved the direct reaction of DAST with the protected L-methionine sulfoxide. Their findings were that although isolable in the protected state, the hydrolytic instability of the FH_2C-functionality ultimately led to its decomposition.



L-monofluoromethionine

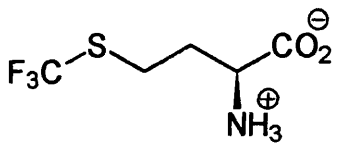
A similar decomposition observation was cited in an earlier paper by A.F. Janzen and coworkers (79). In their paper, describing the monofluorination of

protected methionine with xenon difluoride, they found both aqueous and non-aqueous solutions of HF, HCl or TFA led to the decomposition of L-monofluoromethionine even when in the protected state condition.

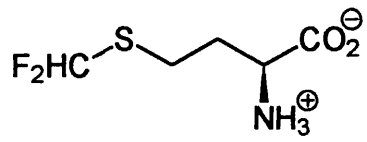
It was at this point in the historical development of fluorinated analogues of methionine that that we decided to pursue the synthesis and biological testing of trifluoromethionine and other related fluoro analogues of L-methionine.

5.5 Initial aims of this research project

Our experimental starting point was to repeat the syntheses of Houston and Honek, via a modified and higher yielding route, and biologically test the difluoro and trifluoro analogues of L-methionine for growth inhibiting properties and or selective toxicity in *Trichomonas vaginalis.*



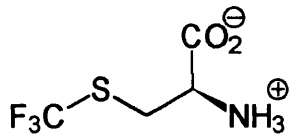
L-trifluoromethionine



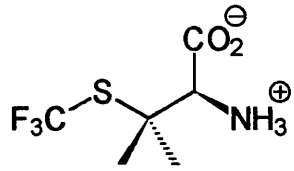
L-difluoromethionine

Subsequent to embarking on a programme of chemical synthesis and biological research, a communication was published (80), pertaining to a new and more convenient method of preparation of the trifluoromethyl analogues of methionine, cysteine and penicillamine. This work, carried out by V. Soloshonok and coworkers, involves the direct trifluoromethylation under U.V. irradiation of the unprotected and enantiomerically pure amino acid with trifluoromethyliodide.

In this communication Soloshonok and coworkers make no reference to the biological activity of any of the three compounds. It was decided to repeat their methods of preparation to synthesise and biologically test L-trifluoromethyl cysteine and D-trifluoromethyl penicillamine.



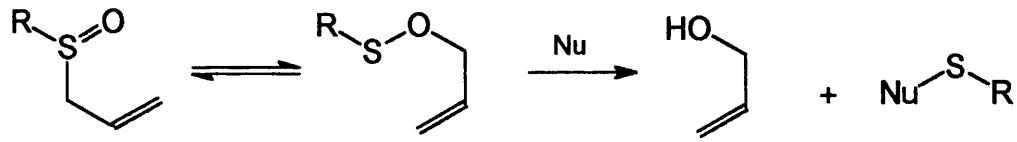
L-trifluoromethyl cysteine



D-trifluoromethyl penicillamine

5.6 Enzyme inactivation via [2,3]-sigmatropic rearrangement

In 1979 C. Walsh and R.A. Firestone published details of a novel mechanism based enzyme inactivation strategy (81) . Their proposal was to invoke a mechanism based enzyme inactivation through inducing a 2,3-sigmatropic rearrangement of an allyl sulfoxide to an allyl sulfenate ester in the enzyme active site. The allyl sulfenate ester, which is highly reactive to nucleophilic addition, would then serve to derivatise the target enzyme. As a result of derivatisation the enzyme is inactivated.

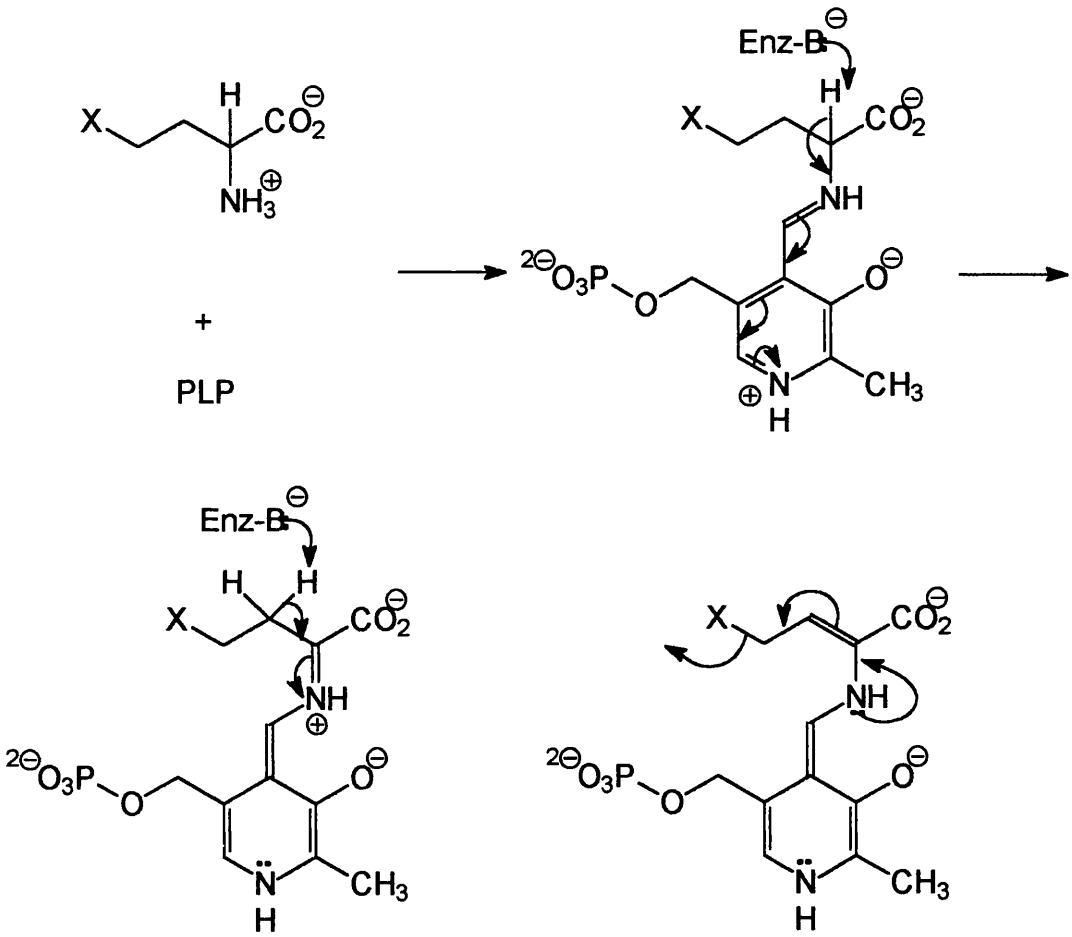


allyl sulfoxide → allyl sulfenate ester rearrangement

In considering potential substrates of the type imagined, Walsh and Firestone knew they would need to design a reagent such that the allyl sulfoxide was only generated in the active site of the targeted enzyme. They designed a halo (X) sulfoxide precursor which could undergo enzyme mediated loss of HX to "uncover" an allylic double bond. Their proposed reaction pathway would then require a minimal two step process to take place before the substrate could reach its 'suicide activation' chemistry.

Pyridoxal phosphate dependent enzymes catalyse the elimination of good leaving groups at the γ-carbon of their amino acid substrates and both

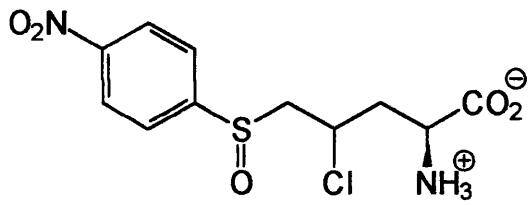
methionine-γ-lyase and cystathionine-γ-synthetase require bound pyridoxal phosphate cofactors to facilitate elimination. Therefore it is not surprising that Walsh and Firestone decided the bacterial enzymes methionine-γ-lyase and cystathionine-γ-synthetase would be likely candidates for generating an allyl sulfoxide in the enzyme active site by HX elimination.



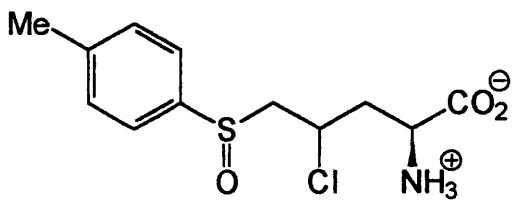
enzymatic pyridoxal phosphate cofactors
facilitating γ-elimination of a good leaving group (X)

Walsh and Firestone synthesised the amino acids 2-amino-4-chloro-5-(*p*-nitrophenylsulfinyl) pentanoic acid and 2-amino-4-chloro-5-(*p*-tolylsulfinyl)

pentanoic acid and demonstrated, via stoichiometric labelling with ^3H, that γ-chloro elimination will generate an enzyme bound allyl sulfoxide. The key 2,3 sigmatropic rearrangement of the allyl sulfoxide to the allyl sulfenate ester then inactivates both methionine-γ-lyase and cystothionine-γ-synthetase via nucleophilic capture of an active site enzyme residue.



2-amino-4-chloro-5-(*p*-nitrophenylsulfinyl) pentanoic acid



2-amino-4-chloro-5-(*p*-tolylsulfinyl) pentanoic acid

Walsh and Firestone's studies showed that although 2-amino-4-chloro-5-(*p*-nitrophenylsulfinyl) pentanoic acid effected inactivation of the enzymes methionine-γ-lyase and cystothionine-γ-synthetase, 2-amino-4-chloro-5-(*p*-tolylsulfinyl) pentanoic acid did not. Walsh and Firestone postulated that in the case of the *p*-tolylsulfinyl compound, an unfavourable equilibrium shift for the sulfoxide → sulfenate ester rearrangement might be the cause. Their postulation was in concurrence with a report by K. Mislow (82) which detailed the observation that although both *p*-tolyl and *p*-nitrophenyl allyl sulfoxides exist almost exclusively in the sulfoxide form at equilibrium (>99%), the rates at which they undergo reversible sigmatropic rearrangements are very different. Equilibration of *p*-nitrophenyl allyl sulfoxides could be studied at 7°C, while studies on the equilibration of *p*-tolyl allyl sulfoxide required temperatures in the

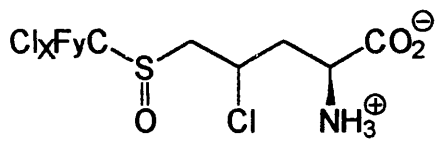
region of 40°C - 60°C. Since Walsh and Firestone's enzyme inhibition studies were conducted at 37°C one can reasonably assume that the lack of inhibiting activity displayed by 2-amino-4-chloro-5-(*p*-tolylsulfinyl) pentanoic acid was a result of unfavourable equilibrium kinetics.

There is some evidence to suggest that the presence of a strongly electron-withdrawing group attached to the sulfur will help shift the equilibrium towards formation of the sulfenate (82).

5.7 Synthesis of related compounds for enzyme inactivation via an allyl sulfoxide → allyl sulfenate ester rearrangement

Building on the work of Walsh and Firestone (81) it was decided to repeat the synthesis of 2-amino-4-chloro-5-(*p*-tolylsulfinyl) pentanoic acid and biologically test this compound in *Trichomonas vaginalis*.

Taking account of what has been previously postulated about equilibrium shifts and strongly electronegative substituents attached to the sulfur, it was decided to attempt the synthesis of some analogous chloro- and fluoro-compounds for further chemical understanding and biological testing.



2-amino-4-chloro-5((*x*)chloro(*y*)fluorosulfinyl) pentanoic acid

(X and Y are variables between 1 and 3, mono and tri)

It was hoped that in addition to creating a more favourable equilibrium shift in the direction of formation of the sulfenate ester in the active site, the presence of fluorine in the molecule would offer i) an improved overall yield of the desired compound via preferred addition during synthesis of the corresponding sulphenyl chloride across the double bond of the protected L-allylglycine from

which it is prepared and ii) convey an increased level of toxicity to the bacterial enzyme through improved lipophilicity and increased susceptibility to nucleophilic attack by an active site enzyme residue.

RESULTS AND DISCUSSION

Chapter 6

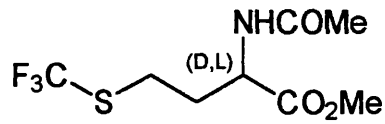
Fluorinated Analogues of L-Methionine

L-Trifluoromethionine

As outlined in the introduction, the experimental starting point had been to repeat the synthetic procedures of Houston and Honek (78), via a modified and higher yielding route. It was then intended to biologically test L-trifluoromethionine, as a fluorinated analogue of L-methionine, for growth inhibition and / or selective toxicity in the anaerobic protozoan flagellate *Trichomonas vaginalis.* L-methionine is a known substrate of *T. vaginalis* .

However in order to synthesise L-trifluoromethionine by the chosen literature method, one first needed to prepare, then isolate, N-acetyl-D,L-trifluoromethionine methyl ester from the starting material N-acetyl-D,L-homocysteine thiolactone.

6.1 Synthesis of N-Acetyl-D,L-trifluoromethionine methyl ester



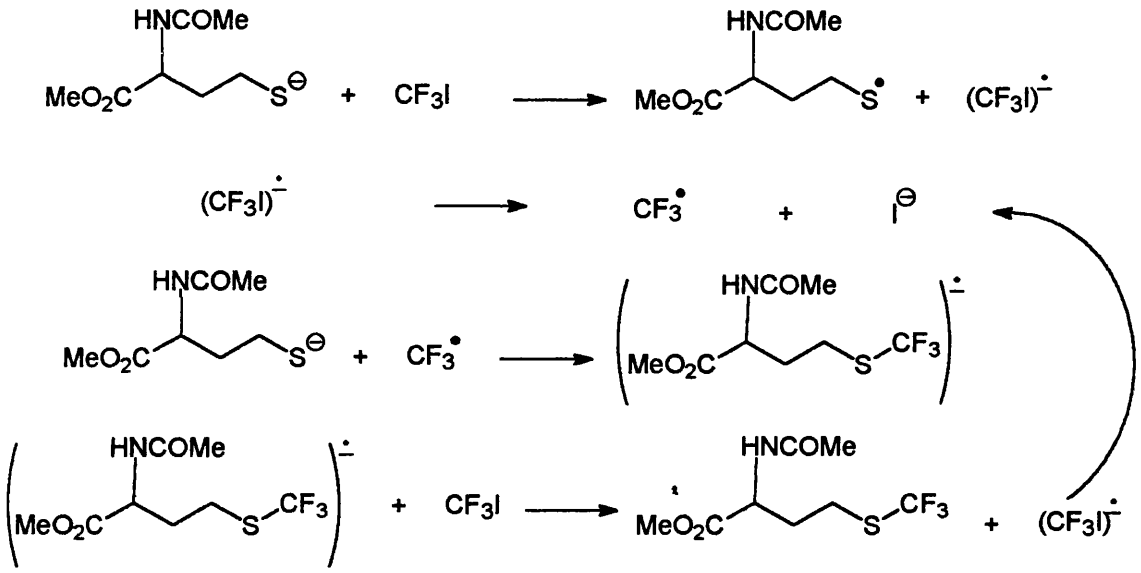
Formation of N-acetyl-D,L-trifluoromethionine methyl ester followed an adaptation of M.E. Houston and J.F. Honek's literature procedure (78). Changes made to the literature procedure were required to achieve good yields of the trifluorinated intermediate product. (In our preliminary attempts following the literature procedure, yields of only 15-20% were realised versus the 52-61% quoted in the literature).

Since ring opening and esterification of N-acetyl-D,L-homocysteine thiolactone, by sodium methoxide in methanol, was reaching completion after 30 mins (confirmed by TLC) the low yielding step in the reaction sequence was identified as the trifluoromethylation step. The literature procedure of bubbling an excess of CF_3I through the reaction mixture at 0°C was evidently not proving a satisfactory method in the equipment set up. Initial attempts to rectify the poor yield involved significantly reducing the flow rate of the CF_3I gas through the reaction mixture. (This was achieved by reducing the incoming flow of $N_{2(g)}$ which would displace the $CF_3I_{(g)}$ from the holding vessel). However, this did not result in significantly improved yields. The CF_3I gas (b.p. -22.5°C) could still be seen bubbling out of the reaction mixture almost immediately after entering it.

Since CF_3I undergoes S_N2 reactions with great difficulty, if at all, due to polarisation of the C–I bond ($F_3C^{\delta-}\!\leftarrow\!I^{\delta+}$) (83), the trifluoromethylation step is believed to follow a radical single electron transfer (SET) type reaction mechanism. Radical ion initiation was achieved via U.V. irradiation. Since this type of reaction is independent of temperature, the next plan to improve the reaction yield involved significantly reducing the reaction temperature during the trifluoromethylation step. The reaction temperature was lowered from 0°C to -50°C. Following this change to the literature method, the reaction yield improved to 64%. This was an overall 3% improvement on the literature value (61%) and a 44% improvement on previous efforts.

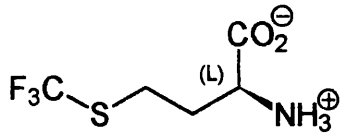
Having shown that a 50°C reduction in temperature does not affect the reaction path followed, this provides further support for an $S_{RN}1$ mechanism.

$S_{RN}1$ mechanism



IR, ^{1}H NMR, ^{13}C NMR, ^{19}F NMR spectra, m/z and micro analysis all support the formation of N-acetyl-D,L-trifluoromethionine methyl ester.

6.2 Synthesis of L-Trifluoromethionine



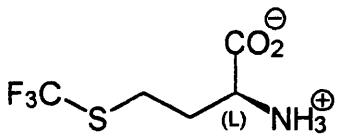
Formation of L-trifluoromethionine followed the literature procedure of M.E. Houston and J.F. Honek (78). Complete hydrolysis of the methyl ester of N-acetyl-D,L-trifluoromethionine methyl ester was achieved with aqueous NaOH in methanol after 50 mins at 0°C. Stereoselective amine de-protection to afford the L-amino acid only was effected by the enzyme porcine kidney acylase in an aqueous solution, at pH 7.5 and 37°C. Formation of the free amino acid was confirmed by the visualising of a lilac colouration upon treatment with ninhydrin spray in TLC spot tests. The reaction afforded a 37.7% yield of

L-trifluoromethionine (75.4% based on D,L mixture) compared with the literature value of 42.6%.

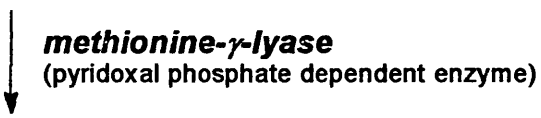
^{1}H NMR, ^{13}C NMR, ^{19}F NMR spectra, m.p., $[\alpha]_D$ and m/z all support the formation of L-trifluoromethionine.

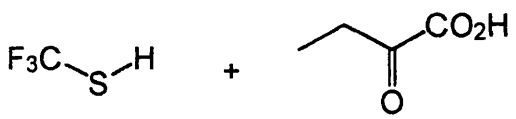
6.3 Biological activity of L-Trifluoromethionine

G.H. Coombs and associates were able to show that L-trifluoromethionine is a growth inhibitor of *Trichomonas vaginalis* (page 168) and a substrate for methionine-γ-lyase purified from *T. vaginalis* (84). Their biological testing demonstrated that during the enzymic reaction, L-trifluoromethionine undergoes an elimination reaction to produce α-ketobutyrate (84). Trifluoromethane thiol, which would also be a product in this reaction pathway, was not detected and this was presumably due to its rapid non-enzymatic decomposition to carbonothionic difluoride.



L-trifluoromethionine

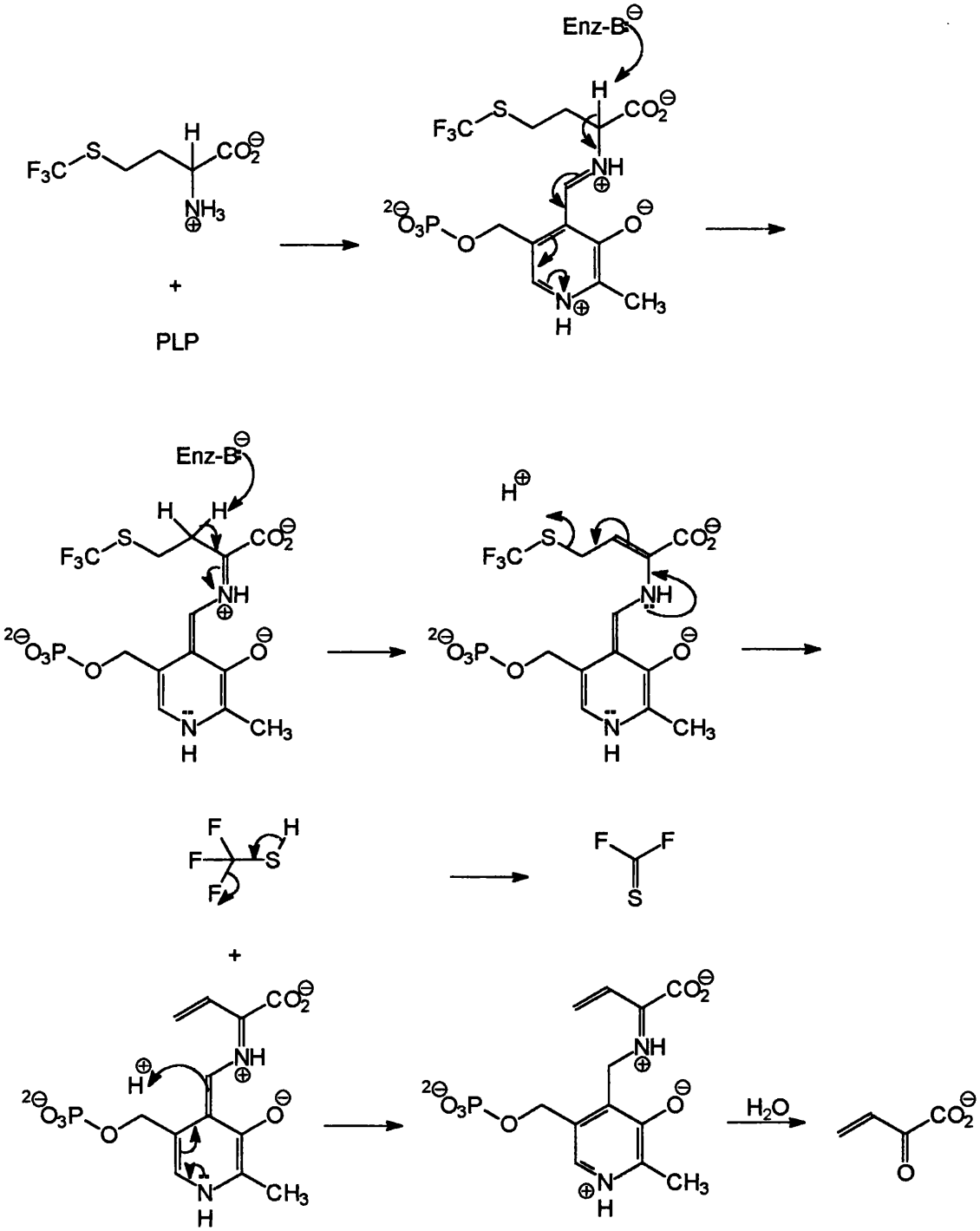




trifluoromethane thiol α-ketobutyrate

Carbonothionic difluoride

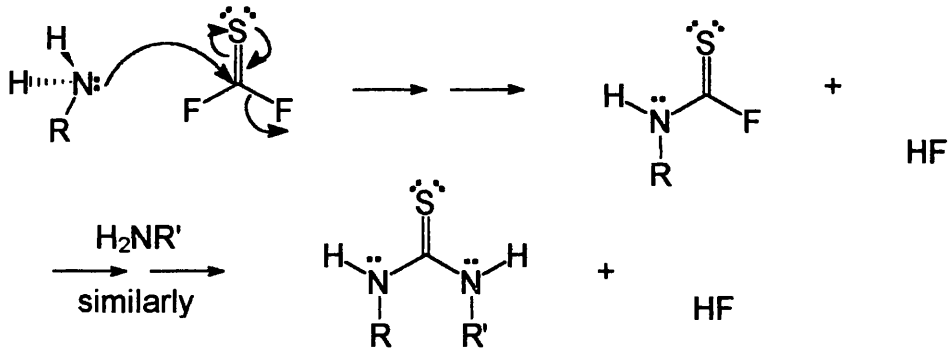
The decomposition of trifluoromethane thiol to carbonothionic difluoride had in 1983 been postulated by T.A. Altson and H.J. Bright in reference to the interaction of a variety of pyridoxal phosphate dependent enzymes with trifluoromethionine (85).



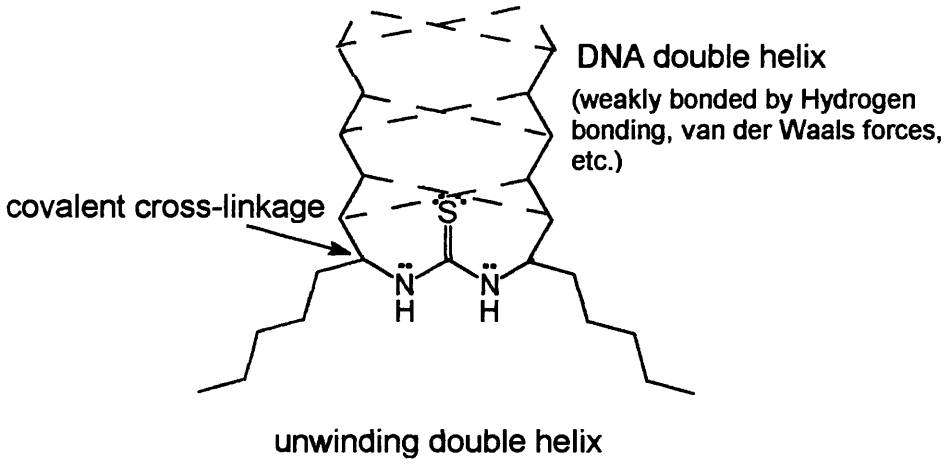
Proposed mechanism for the interaction between L-trifluoromethionine and the pyridoxal phosphate dependent enzyme methionine-γ-lyase

If carbonothionic difluoride were released into the active site of the enzyme it would be susceptible to attack by a nearby nucleophilic enzyme residue. Nucleophilic attack by an enzyme residue on carbonothionic difluoride would result in the formation of a covalent bond which could led to irreversible inactivation of the enzyme and ultimately could lead to the death of the organism.

In addition to being susceptible to nucleophilic attack by an active site enzyme residue, it has been suggested (85) that carbonothionic difluoride could also act as a possible DNA cross-linking agent. Following nucleophilic attack by different amine residues positioned on opposite strands of the DNA double helix and elimination of HF, carbonothionic difluoride could effectively create a series of covalent bonds which would form a bridge between opposite sides of the DNA double helix. If this were the case then the process of DNA replication would be halted by the inability of the double helix to fully unwind.



H_2NR and H_2NR' represent amine residues on opposite strands of the DNA double helix.



Schematic representation of the possible DNA cross-linking effect of carbonothionic difluoride.

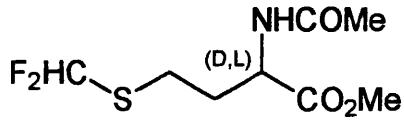
In addition to biological tests on *T.vaginalis,* L- Triflurомethionine was tested for growth inhibiting properties in the other trichomonads; *T. foetus*, *T. augusta* and *T. pyriforris.* While *T. augusta* showed reduced growth, when tested under a variety of drug concentrations, very little and no growth inhibiting capabilities were observed for *T. foetus* and *T. pyriforris* respectively. In *T. augusta* although growth inhibition is observed it is not halted, even at high drug concentration. This suggest that although L-trifluoromethionine inhibits *T. augusta* it does so via a different route to that observed in *T. vaginalis.* Collectively these results suggest that the enzyme L-methionine-γ-lyase is present in only *T.vaginalis* and absent in the other trichomonads tested.

For comparison purposes L-trifluoromethionine was tested in a range of other organisms including; *G. lamblia, E. invadins, C. fasciculata, H.m. muscanum, H.m. ingenoplastis, L. donovani, L. tarenolae, L. mexicana, L. major* and *macrophage*. However L-trifluoromethionine showed significant growth reduction in only *L. donovani* (after 72 hr at ≥10 μg ml^{-1} drug concentration) and in *C. fasciculata* (after 120 hr at all drug concentrations). Although for both organisms nil growth was never observed. Again the observation that growth was not halted for any of the above organism suggests that none of them contained methionine-γ-lyase.

L-Difluoromethionine

For biological specificity and toxicity comparison testing versus L-trifluoromethionine, the analogue L-difluoromethionine was prepared. Again the first step in the synthetic pathway was the synthesis and isolation of an intermediate product, N-acetyl-D,L-difluoromethionine methyl ester, prepared from the starting material N-acetyl-D,L-homocysteine thiolactone.

6.4 Synthesis of N-Acetyl-D,L-difluoromethionine methyl ester



Formation of N- acetyl-D,L-difluoromethionine methyl ester followed an adaptation of M.E. Houston and J.F. Honek's literature procedure (78).

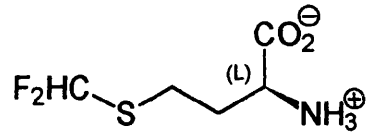
Ring opening and esterification was achieved as previously described in the formation of N-acetyl-D,L-trifluoromethionine methyl ester. Changes to the literature procedure were made during the difluoromethylation step to cut down on the amount of difluorochloromethane required and as a consequence improve the overall economic feasibility of the reaction.

In the difluoromethylation step the literature procedure describes bubbling chlorodifluoromethane through the reaction mixture at 60°C, for a period of 10 h. If one were to do this one would use up a great deal of, if not all, the available difluorochloromethane *(Freon 22)* supply. In considering the b.p. of *Freon 22* (-40.7°C) and the literature reaction temperature (60°C), which is more than 100°C in excess of *Freon 22's* b.p., it was decided to alter the reaction procedure in an attempt to limit the amount of unreacted *Freon 22* gas escaping over the course of the reaction. However it was decided to do this without changing the temperature of the reaction. By taking advantage of the fluorine chemistry vacuum line one had at one's disposal, it was decided to employ a pressurised closed system approach to this reaction procedure. On

the vacuum line an equimolar amount of *Freon 22* was condensed directly into an air evacuated pressure bottle. The pressure bottle having previously been charged with the sodium salt of N-acetyl-D,L-homocysteine methyl ester and methanol. The remainder of the reaction followed the literature method, although in an increased pressure environment (the pressure inside the reaction bottle was estimated to be around 2 atmospheres). The reaction proceeded via the S_N2 displacement of chlorine to afford a 55% yield of N-acetyl-D,L-difluoromethioine methyl ester. This yield, although lower, is within comparable limits of the literature value of 61%.

IR, ^1H NMR, ^{13}C NMR, ^{19}F NMR spectra and m/z all support the formation of N-acetyl-D,L-difluoromethionine methyl ester.

6.5 Synthesis of L-Difluoromethionine



Formation of L-difluoromethionine followed the literature procedure of M.E. Houston and J.F. Honek (78) and again employed the enzyme porcine kidney acylase to effect stereoselective amine de-protection and afford the L-amino acid only. L-Difluoromethionine was isolated in 41.1% overall yield (82.2% based on D,L mixture) compared with a literature value of 38.1%. Again formation of the free amino acid was confirmed by visualising of a lilac colouration upon treatment with ninhydrin spray in TLC spot tests.

^1H NMR, ^{13}C NMR, ^{19}F NMR spectra, m.p., $[\alpha]_D$, m/z and micro analysis all support the formation of L-difluoromethionine.

6.6 Biological Activity of L-Difluoromethionine

G.H. Coombs and associates were able to demonstrate that L-difluoromethionine does act as a growth inhibitor of *T. vaginalis*, although it is

not as potent as L-Trifluoromethionine. (The raw data for this biological testing result could not be located at the time of writing up and is therefore absent from this thesis).

If one makes the assumption that L-difluoromethionine interacts with the enzyme methionine-γ-lyase of *T. vaginalis* via the same transamination route as found for L-trifluoromethionine, then the products formed from L-difluoromethionine will be α-keto butyrate and carbonothionic monofluoride. Carbonothionic monofluoride if released into the active site of the enzyme will, as has been postulated for carbonothionic difluoride (85), also be susceptible to attack by a nucleophilic enzyme residue. Again this could result in irreversible enzyme inactivation and ultimately, if present at a high enough concentration, could lead to the death of the organism.

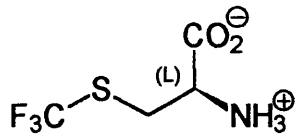
Carbonothionic monofluoride would however not be able to function as a DNA cross-linking agent as has been suggested for L-trifluoromethionine. However that is not to say a reaction between carbonothionic monofluoride and an amine residue on one of the strand of the unwinding DNA double helix could is not a possibility. If this interaction were to occur then carbonothionic monofluoride could still interfere with the DNA replication process by instigating a DNA code miss-read.

Chapter 7

Fluorinated Analogues of L-Cysteine and D-Penicillamine

7.1 Synthesis of L-Trifluoromethyl cysteine

Again for biological specificity and toxicity comparison testing versus L-trifluoromethionine, the analogue L-trifluoromethyl cysteine was prepared directly from the unprotected amino acid L-cysteine.



Formation of L-trifluoromethyl cysteine followed the procedure of V. Soloshonok and co-workers (80) with only minor changes to improve the overall yield;

i) The temperature of the reaction flask was maintained at -50°C for the duration of the reaction versus -35°C in the literature, ii) the molar excess of CF_3I was increased from 2M to 2.60M (this was driven by the capacity of the vacuum line flask) and iii) the irradiation time was extended from 1h in the literature procedure to 1.5h.

Trifluoromethylation at the S position of the unprotected L- aminoacid was achieved following initiation by U.V. irradiation using CF_3I in a radical $S_{RN}1$ reaction (as described earlier). The L-trifluoromethyl cysteine product was isolated in 73.2% yield versus a literature value of 75%.

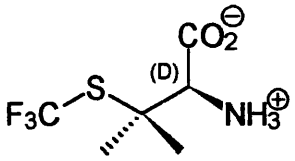
^1H NMR, ^{13}C NMR, ^{19}F NMR spectra, m.p., $[\alpha]_D$, m/z and micro analysis all support the formation of L-trifluoromethyl cysteine.

7.2 Biological activity of L-Trifluoromethyl cysteine

G.H. Coombs and associates (page 168) were able to demonstrate that L-trifluoromethyl cysteine does act as a growth inhibitor of *Trichomonas vaginalis*. However in further tests L-trifluoromethyl cysteine was found to be effective at reducing / halting growth in a wide range of organisms (pages 168 - 173, 175). This finding suggest that L-trifluoromethyl cysteine may not be as enzyme specific in its mode of action as L-trifluoromethionine. This result could also suggest that the hydrocarbon chain length (distance between the α-amino acid moieties and the thiomethyl moiety) is crucial to the methionine-γ-lyase enzyme recognition process.

7.3 Synthesis of D-Trifluoromethyl penicillamine

Again for biological specificity and toxicity comparison testing versus L-trifluoromethionine, the analogue D-trifluoromethyl penicillamine was prepared directly from the unprotected amino acid D-penicillamine.



Formation of D-trifluoromethyl penicillamine followed the procedure of V. Soloshonok and co-workers (80) with only minor changes. i) the molar excess of CF_3I used was 2.88M versus 2.0M in the literature procedure, and both ii) the

temperature and iii) the reaction times were as described above in the synthesis of L-trifluoromethyl cysteine.

Deprotonation of the thiol and trifluoromethylation at the sulfur of the unprotected L- amino acid were achieved as described for L-trifluoromethyl cysteine. The reaction afforded a 65.7% yield of D-trifluoromethyl penicillamine versus a literature value of 61%.

^{1}H NMR, ^{13}C NMR, ^{19}F NMR spectra, m.p., $[\alpha]_D$, and m/z all support the formation of D-trifluoromethyl penicillamine.

7.4 Biological activity of D-Trifluoromethyl penicillamine

G.H. Coombs and associates (page 176) were able to demonstrate that D-trifluoromethyl penicillamine does not act as a growth inhibitor of *Trichomonas vaginalis*. This lack of inhibiting abilility may be due either to: i) the steric bulk afforded by the two CH_3 groups attached to the β carbon inhibiting D-trifluormethyl penicillamine from being recognised as a substrate by the enzyme methionine -γ-lyase or ii) the absence of any hydrogens bonded to the β carbon inhibiting this compound from taking part in the transamination process. If this were the case then D-trifluoromethyl penicillamine could reversibly attach and then detach unchanged from the active site of methionine-γ-lyase, thus exerting no inhibitory effect on this enzyme.

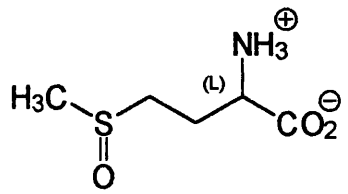
The D stereochemistry of this compound is unlikely to have been responsible for the potential loss of enzyme specificity since the spatial arrangement of the $CO_2^{\ominus}$ group, the $NH_3^{\oplus}$ group and the S containing side chain about the chiral centre remain unchanged compared to L-methionine or any of the already prepared fluorinated analogues.

Chapter 8

Fluorinated Analogues of L-Methionine sulfoxide & L-Methionine sulfone

In attempting to prepare the corresponding sulfoxide and sulfone of L-trifluoromethionine it was hoped to; firstly biologically test whether or not these two compounds were inhibitory substrates for methionine-γ-lyase of *T. vaginalis* and secondly whether oxidation of the sulfur in L-trifluoromethionine to either the sulfoxide or the sulfone had the effect of increasing the metabolic stability and hence potency of this compound.

8.1 Synthesis of L-Methionine sulfoxide



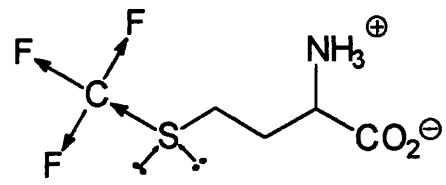
Having been unsuccessful in attempts to directly oxidise trifluoromethionine to trifluoromethionine sulfoxide with either:

i) 30% hydrogen peroxide and glacial acetic acid in ethanol or
ii) treatment with 60% perchloric acid and ammonium molybdate(VI) in water followed by addition of 30% hydrogen peroxide,

One decided to check the validity of the reaction procedure using the non-fluorinated starting material L-methionine. L-Methionine sulfoxide was successfully prepared from the reaction of L-methionine with 30% hydrogen peroxide and glacial acetic acid in ethanol. L-Methionine sulfoxide was isolated in an overall yield of 58.0% compared with the literature value 85.8%. Small amounts of unreacted L-methionine were also recovered.

A characteristic cabbage-like odour, m.p. and m/z all support the formation of L-methionine sulfoxide.

Since these results confirm that the method of oxidation is a reliable one, one can surmise that it must be the presence of the trifluoromethyl group which is impeding the oxidation reaction. Since it is known that the trifluoromethyl group can exert strongly electron withdrawing effects on neighbouring atoms and that the S which one wishes to oxidise is adjacent to the CF_3 group, it is likely that electronic charge is being drawn away from S and towards the CF_3 group. This inductive effect results in a lowered electron density around the sulfur atom. This reduction in electron density then has the effect of reducing the potential for S to undergo oxidation in the above reaction.



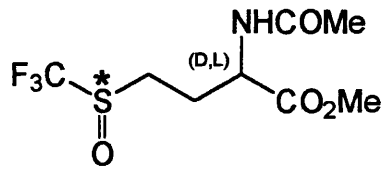
Inductive effect of CF_3 group on adjacent sulfur,

→ indicates the direction of e^- flow

One way to overcome the reduced ability of S to participate in the reaction would be to employ a stronger oxidising agent such as mCPBA, which is what was attempted next.

Since mCPBA is a water sensitive oxidising reagent it was decided to use the previously synthesised and protected form of trifluoromethionine, N-acetyl-D,L,-trifluoromethionine methyl ester, in this reaction. Once one had successfully synthesised the corresponding sulfoxide, it was planned to deprotect the amino acid by the method previously describe for N-acetyl-D,L-trifluoromethionine to give L-trifluoromethionine sulfoxide.

8.2 Synthesis of N-Acetyl-D,L-trifluoromethionine sulfoxide methyl ester



* - new chiral centre

The experimental oxidation procedure followed that described in C. Walsh and R.A. Firestone's paper (81). From the reaction of N-acetyl-D,L-trifluoromethionine methyl ester with mCPBA, one was able to successfully synthesise N-acetyl-D,L-trifluoromethionine sulfoxide methyl ester as a clear oil in an overall yield of 91.6%.

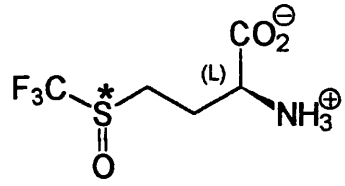
^{1}H NMR, ^{13}C NMR, ^{19}F NMR spectra, m/z and micro analysis all support the formation of N-acetyl-D,L-trifluoromethionine sulfoxide methyl ester.

In particular, the ^{19}F NMR spectrum exhibits 2 signals (-73.61, -73.64) where the starting material shows only 1 signal (-41.9). This experimental data supports the formation of a new chiral centre at the sulfur and the formation of 2 new stereoisomers. The change in chemical shifts supports a reduction in

the electron density around the fluorine atoms and reflects the inductive electron withdrawing effect of the sulfoxide oxygen.

Additional support for the formation of the S=O bond comes from a change in the chemical character, and consequently the chemical shifts, of the atoms adjacent to the sulfur. A shift in the $CH_{2\gamma}$ ^{13}C NMR signal (from 32.5 to 44.7) shows the decrease in the level of electron shielding around this carbon.

8.3 Attempted synthesis of L-Trifluoromethionine sulfoxide



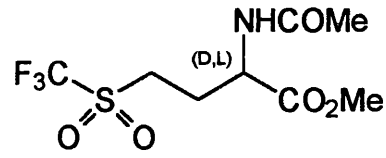
Following the procedure of Houston and Honek (78) for the selective deprotection of N-acetyl-D,L-trifluoromethionine methyl ester, one successfully hydrolysed the methyl ester protecting group with an aqueous solution of sodium hydroxide to form the sodium salt. This reaction, as in previous tests, was monitored by TLC to assure completion. However on overnight treatment of an aqueous solution of the sodium salt with porcine kidney acylase at pH 7.5, 37°C, no free amino acid could be either detected, with ninhydrin spray, or isolated by ion exchange column chromatography.

Considering this was a selective deprotection procedure which had been carried out successfully many times before, this result suggested that either i) the addition of a new chiral centre at sulfur, or ii) the presence of the sulfoxide oxygen; inhibit this intermediate product from being a recognised substrate for the enzyme porcine kidney acylase. To test this theory it was decided to remove the chirality variable by attempting to prepare the corresponding protected sulfone.

L-Trifluoromethionine sulfone

For the reasons explained earlier in the preparation of L-trifluoromethionine sulfoxide, it was decided to use the previously synthesised N-acetyl-D,L,-trifluoromethionine methyl ester in this reaction. Once one had successfully synthesised the corresponding sulfone, it was planned to deprotect the amino acid, as previously describe for N-acety-D,L-trifluoromethionine, to give L-trifluoromethionine sulfone.

8.4 Synthesis of N-Acetyl-D,L-trifluoromethionine sulfone methyl ester

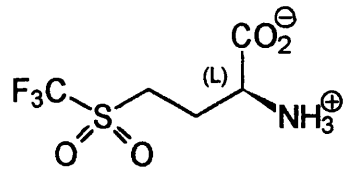


Again following the experimental oxidation procedure described in C. Walsh and R.A. Firestone's paper (81), but with a greater than 2.0M excess of mCPBA, one was able to successfully synthesise N-acetyl-D,L-trifluoromethionine sulfone methyl ester as a clear oil in an overall yield of 87.2%.

^{1}H NMR, ^{13}C NMR, ^{19}F NMR spectra and m/z all support the formation of N-acetyl-D,L-trifluoromethionine sulfone methyl ester.

In particular, the ^{19}F NMR spectrum is exhibiting only one signal (-78.3), which is positioned upfield compared with the starting material signal (-41.9). Again a change in the position of the $CH_{2}\gamma$ ^{13}C NMR signal (shifting downfield from 32.5 for L-methionine to 46.3 for the sulfone) demonstrates a decrease in electron shielding around the $CH_{2}\gamma$ carbon.

8.5 Attempted synthesis of L-Trifluoromethionine sulfone



Again following the procedure of Houston and Honek (78) for the selective deprotection of N-acetyl-D,L-trifluoromethionine methyl ester, one successfully hydrolysed the methyl ester protecting group with an aqueous solution of sodium hydroxide to form the sodium salt. This reaction was monitored by TLC to assure completion. Disappearance of the $CO_2C\underline{H_3}$ signal from the ^1H NMR spectrum also confirmed formation of the sodium salt. However once again, on overnight treatment of an aqueous solution of the sodium salt with porcine kidney acylase at pH 7.5, 37°C, no free amino acid could be either detected, with ninhydrin spray, or isolated on evaporation *in vacuo.*

This result supports the theory that the presence of oxygen, either in the form of the sulfoxide or the sulfone, inhibits this intermediate product from being recognised as a substrate at the active site of the enzyme porcine kidney acylase.

One way to overcome the effect of losing this enzyme specificity would be to prepare only the L-isomer of the protected amino acid, e.g. N-acetyl-L-trifluoromethionine methyl ester, before proceeding to the oxidation step. This would eliminate the need for selective deprotection using porcine kidney acylase in the final step of the reaction. However due to time constraints and higher project priorities this was not attempted.

Chapter 9

Preparation of 2-amino-4-chloro-5-(p-nitrophenylsulfinyl) pentanoic acid

Synthesis of:

9.1. N-Acetyl-D,L-allylglycine

9.2 L-Allylglycine

9.3 N-Boc-L-allylglycine

9.4 N-Boc-L-allylglycine benzhydryl ester

9.4.1 Benzophenone hydrazone

9.4.2 Diphenyl diazomethane

9.4.3 N-Boc-L-allylglycine benzhydryl ester

9.5 4-nitrophenylsulfenyl chloride

9.6 2-N-Boc-4-chloro-5-(p-nitrophenylsulfenyl)pentanoic acid benzhydryl ester

9.7 Sulfenyl chloride addition to carbon-carbon double bonds

9.7.1 - Alkene substituents and their influence on the ring opening of the episulfinium ion

9.7.2 - Sulfenyl chloride substituents and their influence on the ring opening of the episulfonium ion.

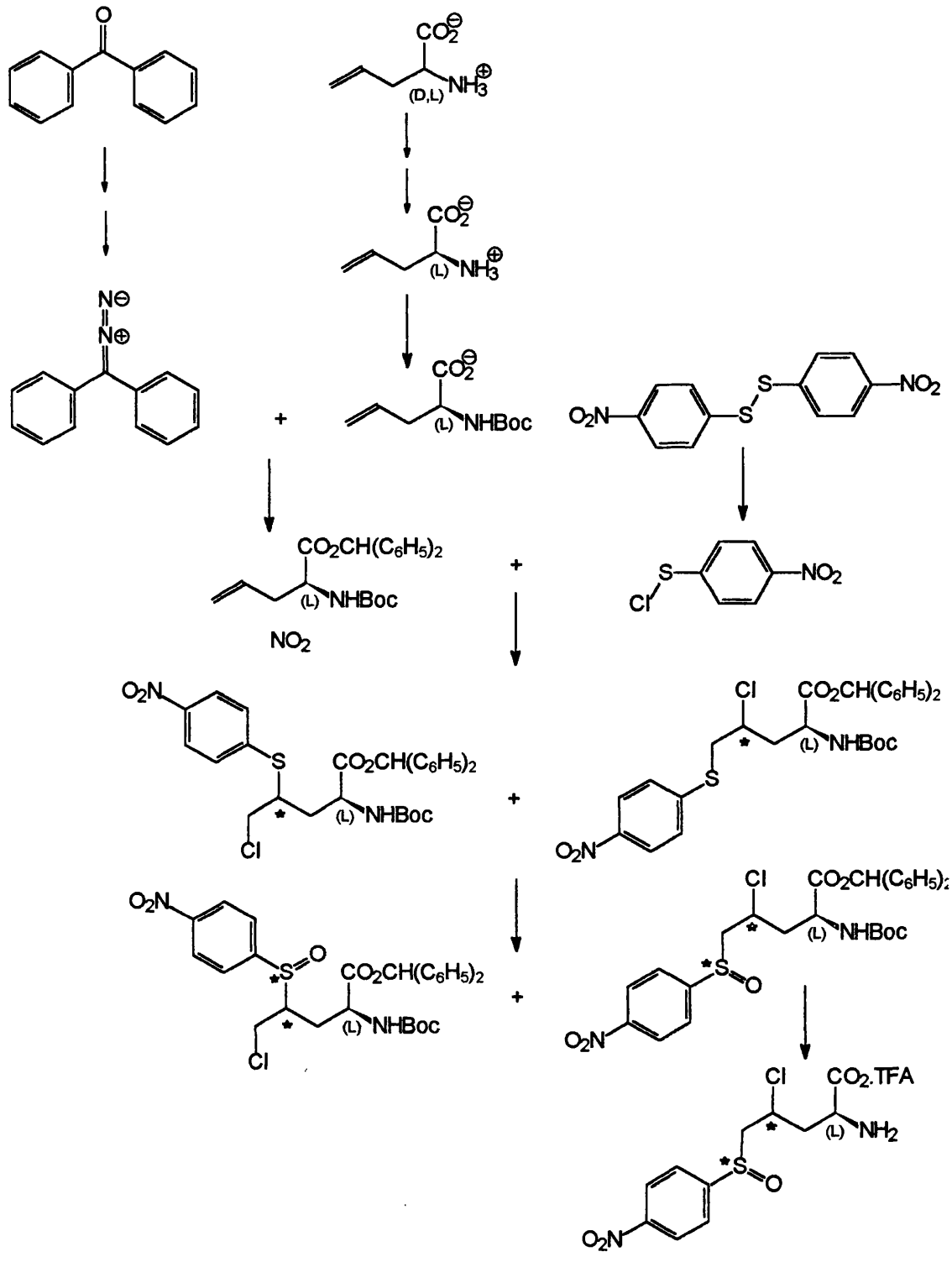
Synthesis of:

9.8 2-N-Boc-4-chloro-5-(p-nitrophenylsulfinyl)pentanoic acid benzhydryl ester

9.9 2-Amino-4-chloro-5-(p-nitrophenylsulfinyl) pentanoic acid.TFA salt

9.10 Biological Activity of 2-Amino-4-chloro-5-(p-nitrophenyl sulfinyl)pentanoic acid

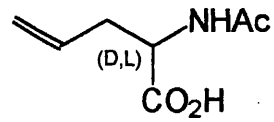
2-Amino-4-chloro-5-(*p*-nitrophenyl sulfinyl) pentanoic acid



Schematic summary of the synthetic route followed in the preparation of 2-amino-4-chloro-5-(*p*-nitrophenyl sulfinyl) pentanoic acid

(★ represents the formation of new chiral centres)

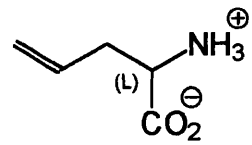
9.1 Synthesis of N-Acetyl-D,L-allylglycine



Following the literature procedure of S. Black and N.G. Wright (86), N-acetyl-D,L-allylglycine was successfully prepared from acetic anhydride and D,L-allylglycine under alkaline conditions. The reaction afforded an overall yield of 74.2% compared with the literature value of 83%.

IR, ^{1}H NMR, ^{13}C NMR spectra, m.p. m/z and micro analysis all support the formation of N-acetyl-D,L-allylglycine.

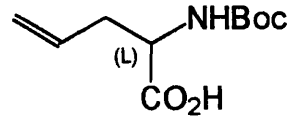
9.2 Synthesis of L-Allylglycine



Following the literature procedure of S. Black and N.G. Wright (86), L-allylglycine was successfully prepared and isolated as white crystals. Selective cleavage of the N-acetyl group was again achieved using the enzyme porcine kidney acylase at 37°C to afford L-allylglycine in an overall yield of 39.4% (78.8% based on the D,L mixture) compared with a literature value of 35%.

IR, ^{1}H NMR, ^{13}C NMR spectra, m.p., $[\alpha]_D$ and m/z all support the formation of L-allylglycine.

9.3 Synthesis of N-Boc-L-allylglycine

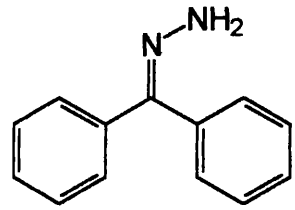


Protection of L-allylglycine using the tertiary butyloxycarbonyl protecting group (Boc) was successfully achieved following the literature procedure of C. Walsh and R.A. Firestone (81). The by-product hydroxyiminophenyl acetonitrile (HO-N=C(CN)C_6H_5) was easily removed from the reaction mixture by extraction with ether. The reaction afforded N-Boc-L-allylglycine in an 81% overall yield from L-allylglycine compared with a literature value of 85.4%.

IR, ^{1}H NMR, ^{13}C NMR spectra, m.p., m/z and micro analysis all support the formation of N-Boc-L-allylglycine.

9.4 N-Boc-L-allylglycine benzhydryl ester

9.4.1 Synthesis of Benzophenone hydrazone



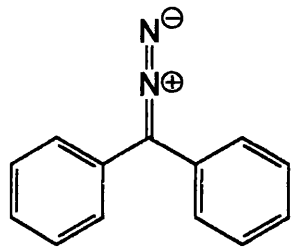
Following the literature procedure in Fieser & Fieser's book of 'Reagents for Organic Chemistry Synthesis' (87), benzophenone hydrazone was successfully prepared from the reaction of benzophenone with hydrazine in an overall yield of 97.4%. This yield is a greater than 10% improvement on the literature value of 87%.

IR, ^{1}H NMR spectra, m.p., m/z and micro analysis all support the formation of benzophenone hydrazone

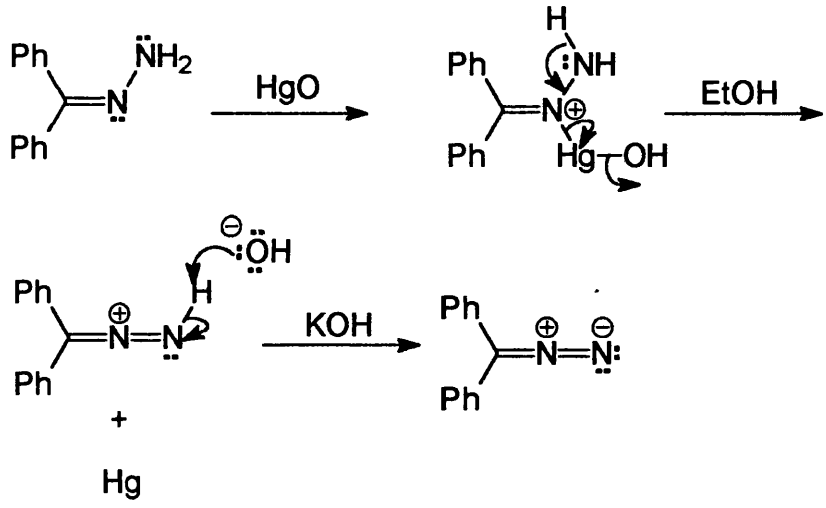
9.4.2 Synthesis of Diphenyldiazomethane

Following the literature procedure in Fieser & Fieser's book of 'Reagents for Organic Chemistry Synthesis' (87), diphenyldiazomethane was successfully prepared from benzophenone hydrazone in an overall yield of 98%, a 9% improvement on the literature value of 89%. This reagent was freshly prepared

before use since it could not be stored for an extended period of time without decomposing (i.e. beyond 2 days in a cool dry place).



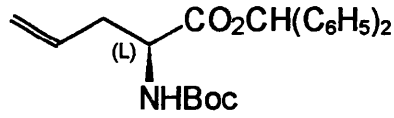
During the course of the reaction mercury(II) oxide is reduced to mercury and water.



Reaction mechanism for the conversion of benzophenone hydrazone to diphenyldiazomethane

IR, ^{1}H NMR spectra, m.p. and the characteristic crimson colour of the crystals formed all support the formation of diphenyldiazomethane.

9.4.3 Synthesis of N-Boc-L-allylglycine benzhydryl ester

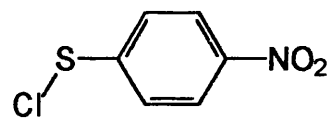


N-Boc-L-allylglycine benzhydryl ester was successfully prepared from the reaction of N-Boc-L-allylglycine with diphenyldiazomethane (liberating N_2 as a by product) by the procedure of C. Walsh and R.A. Firestone (81). The reaction proceeded in an overall yield of 66.7%. This was lower than expected compared with the literature value of 80%.

IR, ^1H NMR, ^{13}C NMR spectra, m.p. and m/z all support the formation of N-Boc-L-allylglycine benzhydryl ester.

9.5 Synthesis of 4-Nitrophenylsulfenyl chloride

After several attempts to accomplish the addition reaction of 4-nitrophenylsulfenyl chloride with the protected L-allylglycine without success, It was decided to consult the literature further before continuing. (The 4-nitrophenylsulfenyl chloride reagent had been bought in 'fresh' from Aldrich, who claimed a purity of 99% in their catalogue). A literature search at this time uncovered a paper published in 1947 by R.A. Turner and R. Connor (88). This paper showed that 4-nitrophenylsulfenyl chloride is only stable for a few hours and should not be stored: It fumes in moist air and cannot be isolated free of the disulfide from which it is prepared. Not surprisingly the decision was made from then on to prepare fresh 4-nitro-phenylsulfenyl chloride prior to use.



4-Nitrophenylsulfenyl chloride

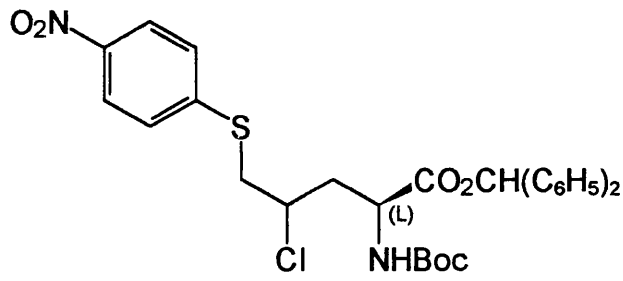
Following the procedure of R. Katakai (89), 4-nitrophenylsulfenyl chloride was successfully prepared from the reaction of 4-nitrophenyl disulphide and liquid chlorine in the presence of a small amount of iodine. Iodine acts as a free-radical catalyst in this reaction via thermally induced homolytic cleavage. The radical ion initiation step is accomplished at a lower temperature with

iodine than would be required for either chlorine or the disulphide since the bond dissociation energy for iodine is much lower (90).

Bond Dissociation energies Kjmol^{-1} at 25°C	
S–S	226
Cl–Cl	242
I–I	151

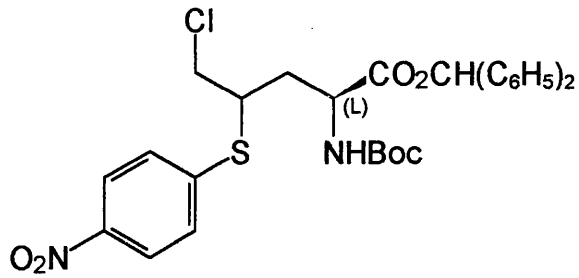
The newly prepared product began to crystallise out towards the end of solvent evaporation step. The product was then stored in the reaction vessel in which it was prepared under N_2 until required. The m.p. of a sample of the crystals was identical to the literature value given for 4-nitrophenyl sulphenylchloride at 48°C. The estimated yield was 95% or above based on the literature procedure and the observation that all the disulphide had dissolved after 10 minutes of reaction time to give a clear orange / yellow solution.

9.6 Synthesis of 2-N-Boc-4-chloro-5-(p-nitrophenylsulfenyl)pentanoic acid benzhydryl ester



Following the literature procedure of C. Walsh & R. A. Firestone, (81), 2-N-Boc-4-chloro-5-(p-nitrophenylsulfenyl) pentanoic acid benzhydryl ester was successfully prepared from the reaction of N-Boc-L-allylglycine benzhydryl ester with *p*-nitrobenzenesulphenyl chloride. However as expected and described in the literature procedure, the product was formed along with its regioisomer, 2-N-Boc-5-chloro-4-(p-nitrophenylsulfenyl)pentanoic acid

benzhydryl ester. This undesirable product was the major product of the reaction. Integration of the.NMR spectrum shows a mixture of the two regioisomers in a 1:10 ratio (desired : undesired).



undesirable regioisomer: 2-N-Boc-5-chloro-4-(p-nitrophenylsulfenyl) pentanoic acid benzhydryl ester

IR, ^1H NMR, spectra, m/z. and microanalysis all support the formation of 2-N-Boc-(4)5-chloro-(5)4-(p-nitrophenylsulfenyl)pentanoic acid benzhydryl ester.

9.7 Sulfenyl chloride addition to terminal carbon - carbon double bonds.

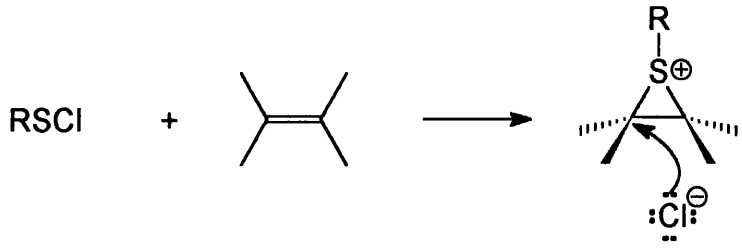
The addition of sulfenyl chlorides across carbon-carbon double bonds has been known for many years and has received considerable attention (91 - 94). A sulfenyl chloride could, in theory, react in at least 3 distinct ways:

1. as a source of electrophilic sulfur (I) or
2. as a source of electrophilic chlorine (II) or
3. as a source of free radicals by homolytic cleavage of the S–Cl bond (III).

$RS^{\delta+}—Cl^{\delta-}$ (I) $RS^{\delta-}—Cl^{\delta+}$ (II) $RS^{\bullet}$ $Cl^{\bullet}$ (III)

Of the three possibilities two have been recognised to date, the occurrence of (I) electrophilic sulfur and (III) free radicals.

Most studies pertaining to the electrophilic addition mechanism have been conducted with 2,4-dinitrobenzenesulfenyl chloride. Due to its outstanding stability it makes a convenient model. This particular reagent undergoes $RS^{\delta+}-Cl^{\delta-}$ polarisation and there is no evidence that the reagent could become the source of Cl^{+} ions (chloronium ions) (95). An investigation by N. Kharasch and associates into the reaction of 2,4-dinitrobenzenesulfenyl chloride with styrene and cyclohexene revealed it to be of 2nd order kinetics (96, 97). All the kinetic data generated to date on sulfenyl chloride addition to alkenes is consistent with but does not require, the formation of an episulfonium ion intermediate (IV).



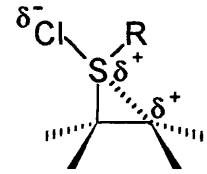
(IV)

episulfonium ion intermediate

Additional support in favour of the formation of an episulfonium ion intermediate is the almost exclusive trans-stereospecific addition observed in the reactions of 2,4-dinitrobenezenesulfenyl chloride with *cis*- and *trans*-2-butenes and norbornane (98 - 100). Particularly strong evidence against a non-rotating open carbonium ion is supplied by the stereospecific integrity of this addition reaction over a wide range of temperatures (101).

However, the almost exclusive Markovnikov orientation reported for the reaction of 2,4-dinitrobenezenesulfenyl chloride with both styrene and propylene suggests that some carbonium ion character does exist in the transition state.

These results are more compatible with the formation of an unsymmetrical episulfonium ion (V).



(V)

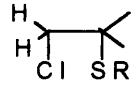
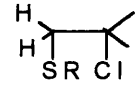
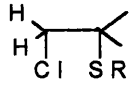
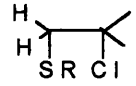
unsymmetrical episulfonium ion intermediate

Previous studies (102) on this topic have clearly shown no single mechanistic route exists and that the course of the reaction is significantly influenced by the nature of the substituents on both the alkene and on the sulfenyl chloride itself.

9.7.1 Influence of the alkene substituents on the ring opening of the episulfinium ion

The high selectivity for anti-Markovnikov oriented products initially observed with alkyl substituted terminal alkenes, and in particular the increase observed with an increase in size of the alkyl substituent, demonstrates that steric factors can significantly influence the nature of the ring opening of the episulfonium ion intermediate (IV). The findings of W.H. Mueller and P.E. Butler ((102) and Table 1.), provide strong evidence for a bridged episulfonium ion intermediate. Their results are completely consistent with nucleophilic attack on the episulphonium ion by the chloride ion. These initially formed alkyl substituted adduct mixtures would generally then undergo rearrangement to form the more stable Markovnikov product in a ~90% ratio at equilibrium.

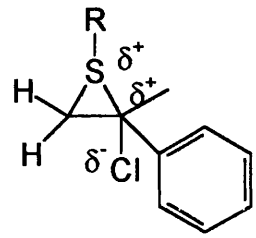
Table 1.

REACTANTS		% ADDUCT RATIO			
		initial		*at equilibrium*	
CH_2=C<	*RSCl*				
CH_2=$CHCH_3$	CH_3SCl	82	18	12	88
CH_2=$C(CH_3)_2$	CH_3SCl	80	20	17	83
CH_2=CHC_6H_5	CH_3SCl	2	98	-	-
CH_2=$C(C_6H_5)_2$	CH_3SCl	10	90	-	-

extracts from reference (102), methane sulfenyl chloride-olefin adducts,

Note: the adduct ratios were calculated from the relative intensity of their characteristic NMR signals.

In contrast to alkyl substituents, phenyl substituents attached to the alkene exhibit predominantly Markovnikov adduct orientated ring opening of the episulfonium ion (VI). This may be explained by a lower transition state energy following nucleophilic attack by the chloride ion on the phenyl substituted carbon versus the transition state energy arising from chloride ion attack on the terminal carbon.



(VI) (R = CH_3)

Markovnikov adduct orientated ring opening of the episulfonium ion when phenyl substituents are attached to the alkene

Stabilisation of the transition state is due to π-bond overlap of the p-orbital of the electron deficient α-carbon with the phenyl ring's π cloud.

The Markovnikov addition products arising from the reaction of phenyl substituted terminal alkenes and sulfenyl chlorides do not show any tendency to rearrange to the anti-Markovnikov orientation (102).

9.7.2 Influence of the sulfenyl chloride substituents on the ring opening of the episulfonium ion.

The nature of the R group on the sulfenyl chloride is of equal importance to the alkene substituents with regard to influencing the orientation of the adduct.

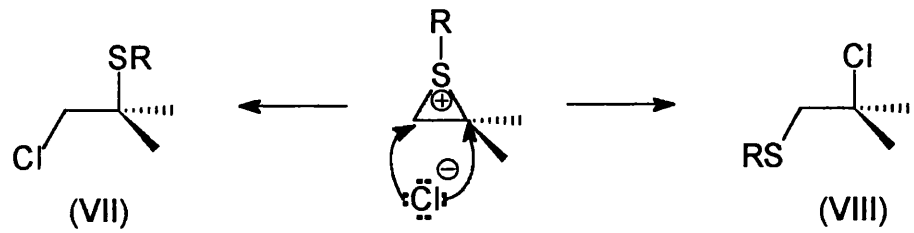
Below (Table 2.) illustrates the effect of the electron withdrawing group $CH_3C(O)S$ on the ring opening of the episulfonium ion in the addition reaction of the substituted sulfenyl chloride to methylpropene. The $CH_3C(O)S$ group tends to destabilise the positive charge on the sulfur of the episulfonium ion. This destabilisation contributes to the development of an electron deficient centre at the substituted carbon. The net result is a predominantly Markovnikov addition product (VIII).

This destabilisation effect is not observed for R groups with low electron-withdrawing influence. This is illustrated when R is CH_3, i.e. methylsulfenyl chloride, where formation of the anti-Markovnikov orientated adduct (VII) is favoured.

Table 2.

	% PRODUCT DISTRIBUTION	
R	**VII anti-Markovnikov**	**VIII Markovnikov**
CH_3	80	20
$CH_3C(O)S$	32	68

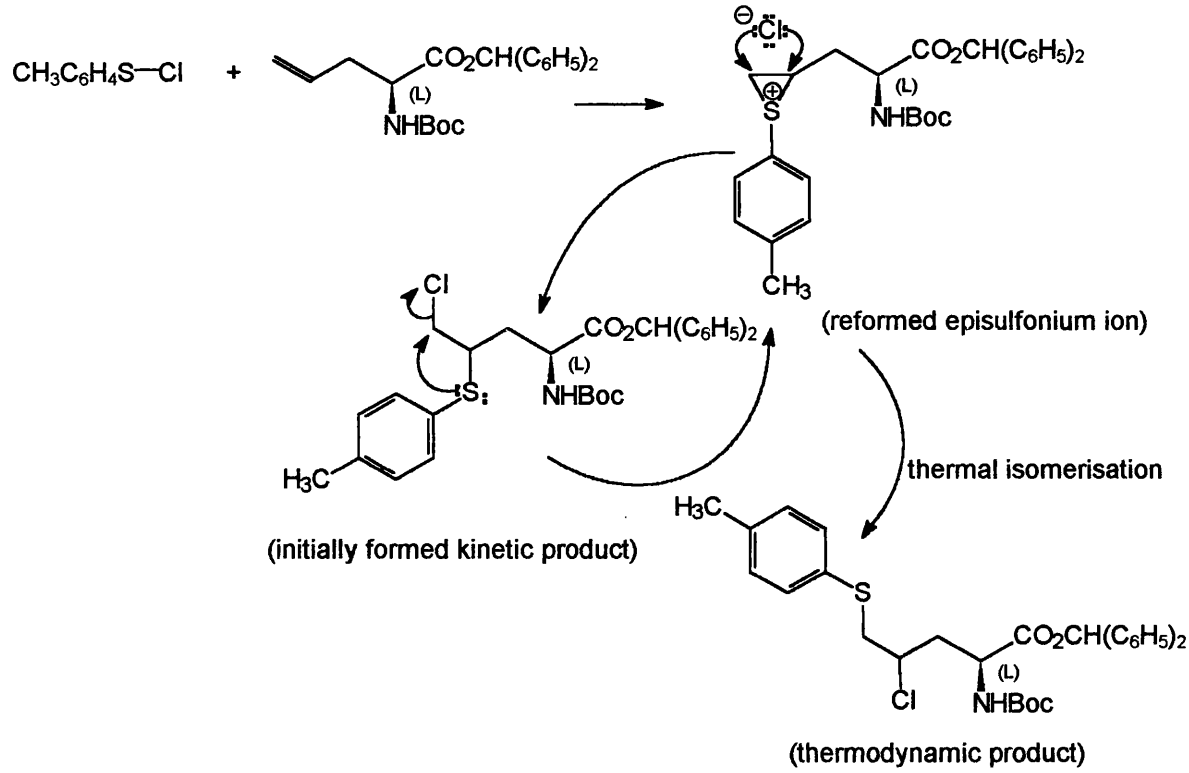
reference 102.



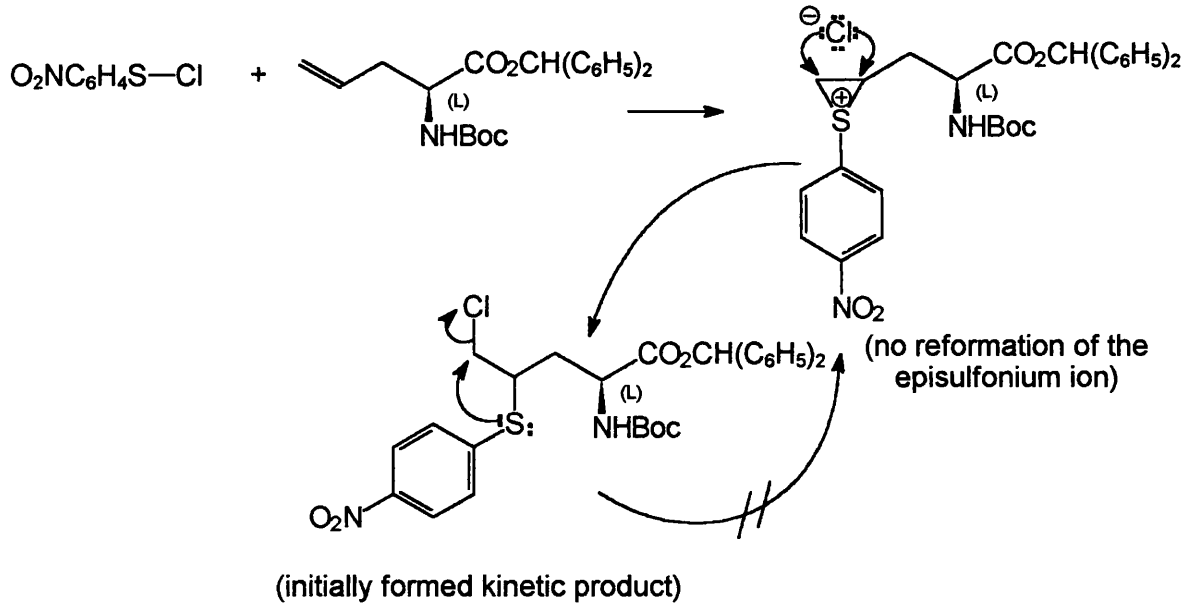
Influence of R on the ring opening of the episulfonium ion in the sulfenyl chloride addition reaction to methylpropene (102)

Further evidence for the important influence of R on the sulfur atom participating in the episulfonium ion comes from the differing tendency for rearrangement of the kinetically controlled (initially formed) adducts and the thermodynamically more stable Markovnikov product.

An example of this is highlighted in an experimental procedure of Walsh and Firestone (81) describing the synthesis of 2-N-Boc-(4)5-chloro-(5)4-(*p*-tolylsulfenyl) pentanoic acid benzhydryl ester. Thermal isomerization of the initially formed 2-N-Boc-5-chloro-4-(*p*-tolylsulfenyl) pentanoic acid benzhydryl ester (kinetic product) to the more stable 2-N-Boc-4-chloro-5-(*p*-tolylsulfenyl) pentanoic acid benzhydryl ester(thermodynamic product) could be achieved after 17h at reflux temperature in chloroform. However, in their separate synthesis of 2-N-Boc-(4)5-chloro-(5)4-(*p*-nitrophenylsulfenyl)pentanoic acid benzhydryl ester, the initially formed 2-N-Boc-5-chloro-4-(*p*-nitrophenylsulfenyl) pentanoic acid benzhydryl ester (kinetic product) could not be thermally isomerised to 2-N-Boc-4-chloro-5-(*p*-nitrophenylsulfenyl)pentanoic acid benzhydryl ester (thermodynamic product). Rearrangement of this regioisomer is much slower and on applying heat the isomerisation is accompanied by degradation. The isomeric rearrangement, which is believed to take place via the reformation of the episulfonium ion intermediate, is less easily accomplished in the presence of an electron withdrawing R group on the sulfur. In the case of 2-N-Boc-5-chloro-4-(*p*-nitrophenyl sulfenyl)pentanoic acid benzhydryl ester, the electronegativity of the 4-nitrophenyl group on the sulfur decreases the availability of the unshared electrons and impairs the sulfur from participating in the intramolecular displacement of the chlorine.

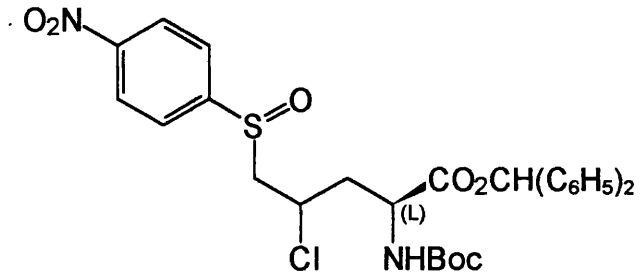


Proposed mechanism for the predominant route of addition for *p*-tolylsulfenyl chloride to N-Boc-L-allylglycine benzhydryl ester



Proposed mechanism for the predominant route of addition for *p*-nitrophenylsulfenyl chloride to N-Boc-L-allylglycine benzhydryl ester

9.8 Synthesis of 2-N-Boc-4-chloro-5-(*p*-nitrophenylsulfinyl)pentanoic acid benzhydryl ester



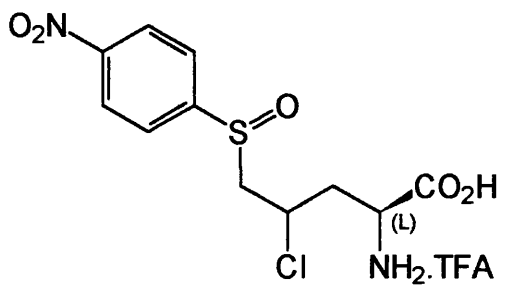
(A)

Following the literature procedure of C. Walsh & R. A. Firestone, (81), 2-N-Boc-4-chloro-5-(*p*-nitrophenylsulfinyl)pentanoic acid benzhydryl ester was successfully prepared by the oxidation of N-Boc-(5)4-chloro-(4)5-(*p*-nitrophenylsulfinyl)pentanoic acid benzhydryl ester with 55% mCPBA in DCM at 0°C. Separation of the desired regioisomer was achieved by repeated flash chromatography to afford an overall yield of 5.5% from the protected L-allylglycine. This yield is lower than the literature value of 9 - 10%.

The isolated product (**A**) exists as 4 diastereoisomers. The creation of a new chiral centre has been brought about by the oxidation of the sulfur to sulfoxide.

The ^{1}H NMR spectrum supports the formation of 2-N-Boc-4-chloro-5-(*p*-nitrophenylsulfinyl) pentanoic acid benzhydryl ester as diastereoisomers.

9.9 Synthesis of 2-Amino-4-chloro-5-(*p*-nitrophenylsulfinyl)pentanoic acid.TFA salt



Following the literature procedure of C. Walsh & R. A. Firestone, (81), 2-amino -4-chloro-5-(*p*-nitrophenylsulfinyl)pentanoic acid was successfully prepared following the simultaneous hydrolysis of the benzhydryl ester and cleavage of the Boc protecting group of 2-N-Boc-4-chloro-5-(*p*-nitrophenylsulfinyl) pentanoic acid benzhydryl ester by the action of anisole and trifluoroacetic acid (TFA). The TFA salt of the amino acid was isolated as a glassy product in an overall yield which exceeded 100% (102%) due to incomplete drying. This compares well with the literature value of 108%.

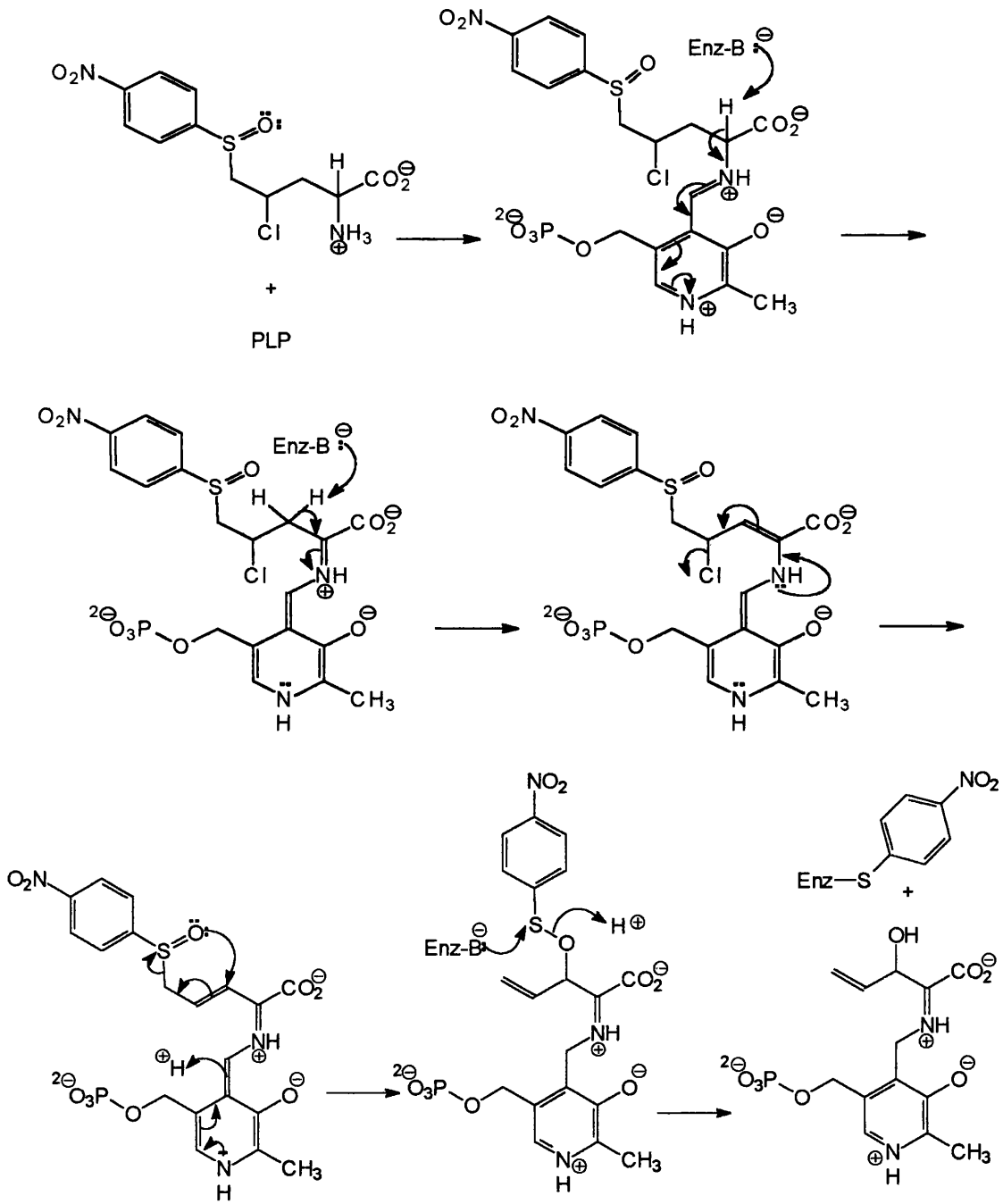
Too rigorous an evaporation of the amino acid TFA salt in aqueous solution tends to lead to a loss of TFA stabilisation. Loss of TFA stabilisation brings about decomposition of the free amino acid through intramolecular displacement reactions.

The ^{1}H NMR and ^{13}C NMR spectra both support the formation of 2-amino-4-chloro-5-(*p*-nitrophenylsulfinyl)pentanoic acid.

9.10 Biological Activity of 2-Amino-4-chloro-5-(*p*-nitrophenylsulfinyl)pentanoic acid

G.H. Coombs and associates were unable to test this product for growth inhibiting properties in *Trichomonas vaginalis*. Unfortunately following analysis and evaporation only 35mgs (0.0065 mmol) of the prepared amino acid TFA salt remained. This was found not to be an adequate amount to conduct sufficient repeatable biological trials on *T. vaginalis*.

However, in the work of Walsh and Firestone (81), 2-amino-4-chloro-5-(*p*-nitrophenylsulfinyl)pentanoic acid was found to be a time dependent irreversible inactivator of methionine-γ-lyase (purified from *Pseudomonas ovalis* not *Trichomonas vaginalis*). Walsh and Firestone were able to demonstrate that the time dependent loss of catalytic activity ultimately led to full inactivation of the enzyme via nucleophilic capture following 2,3-sigmatropic rearrangement of the allylsulfoxide to the allylsulfenate ester.



Pathway proposed by Walsh and Firestone (81) for methionine-γ-lyase inactivation by 2-amino-4-chloro-5-(*p*-nitrophenylsulfinyl)pentanoic acid

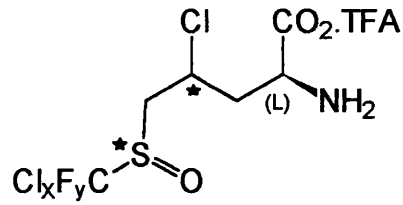
Separation and testing of different sets of diastereoisomers of 2-amino-4-chloro-5-(*p*-nitrophenylsulfinyl)pentanoic acid did not show significantly

different reaction kinetics compared to the results obtained for a preparation containing all four diastereoisomers of (A) (81). This result suggested to Walsh and Firestone that although (L) chirality about the α-carbon is a prerequisite for molecular recognition at the enzyme active site, the stereochemistry about the γ-carbon and sulfoxide are of little importance in achieving enzyme inactivation or the rate at which it is accomplished.

Chapter 10

Preparation of 2-amino-4-chloro-5-(chlorodifluoromethylsulfinyl) pentanoic acid.TFA salt

2-Amino-4-chloro-5-(chlorodifluoromethylsulfinyl)pentanoic acid.TFA salt



(X and Y are numbers between 1 and 3 such that X + Y = 3)

10.1 Identifying an appropriate group of target compounds

The reasons for choosing this group of target compounds, as briefly discussed at the end of chapter 5 of the introduction, are 2-fold:

1. Increased enzyme specificity and toxicity of 2-amino-4-chloro-5 (chlorodifluoromethylsulfinyl)pentanoic acid towards the enzyme methionine-γ-lyase versus the previously synthesised 2-amino-4-chloro-5-(*p*-nitrophenylsulfinyl)pentanoic acid.

i) By introducing fluorine (and or chlorine) into the molecule it was hoped to convey an improved lipid solubility profile. This would result in enhanced absorption, transportation and quicker onset of enzyme inactivation following patient treatment. It could also afford a reduction in the dosage required to effect complete enzyme inactivation.

π measurements (103):

$\pi CH_3 = 0.5$ $\pi CF_3 = 1.07$

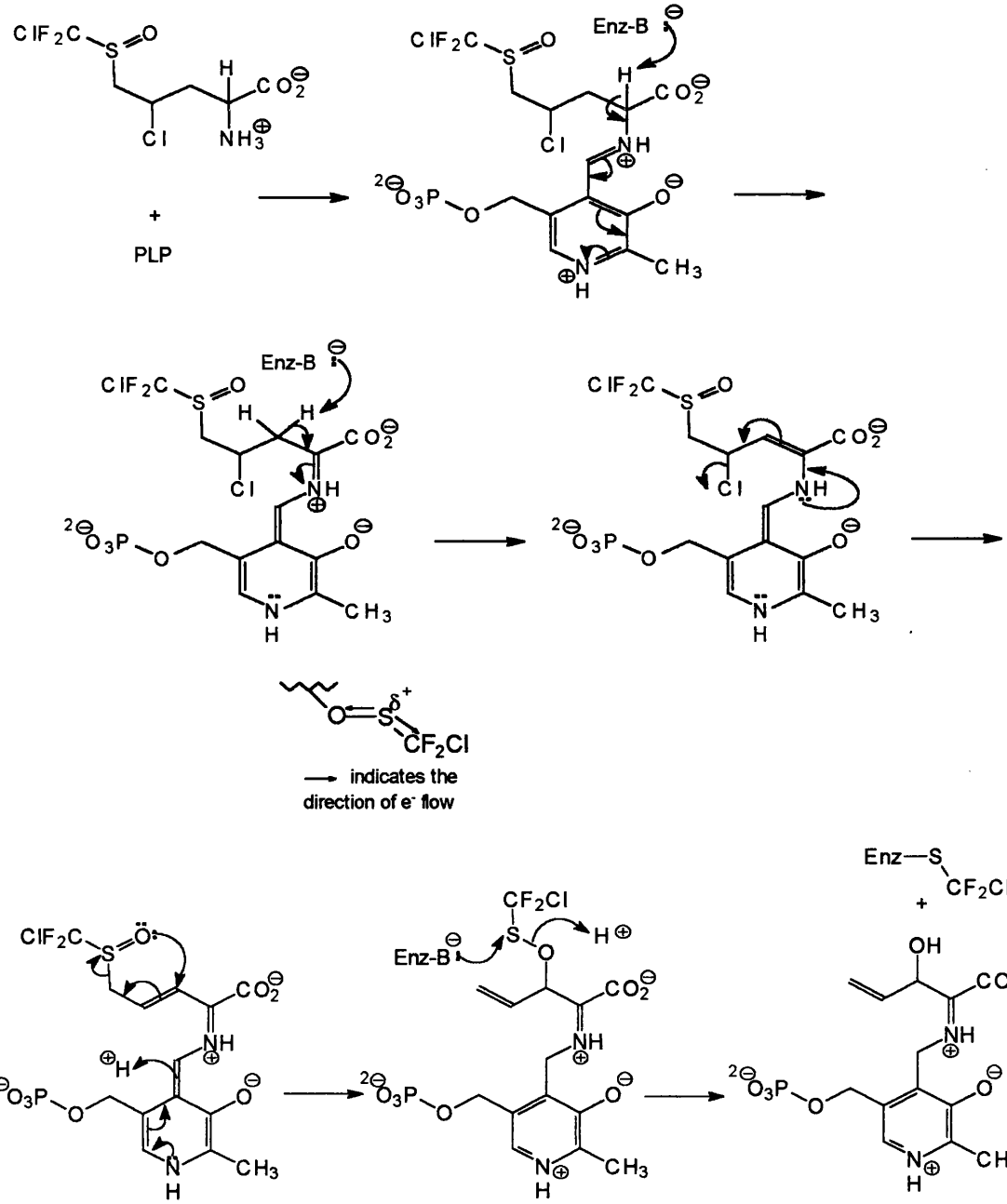
(the π constant is a good indication of lipophilicity, the higher the π value the more highly lipophilic the group).

ii) As a result of replacing the bulky *p*-nitrophenyl sulfinyl group with a fluoro / chloro substituted methyl sulfinyl group, it was hoped to increase the incidence of molecular recognition through more effectively mimicking L-methionine at the enzyme active site. If this replacement did prove to be effective, then an increase in the enzyme specificity and / or drug potency could result.

van der Waals radii (Å) (104):

$CH_3 = 2.0$ $CF_3 = 2.7$

iii) Improved enzyme toxicity through an increased susceptibility of the allylsulfenate to nucleophilic capture by an active site enzyme residue. The electron-withdrawing influence of the chloro / fluoromethyl group is greater than that of *p*-nitrophenyl and will enhance sulfur's electrophilic character.



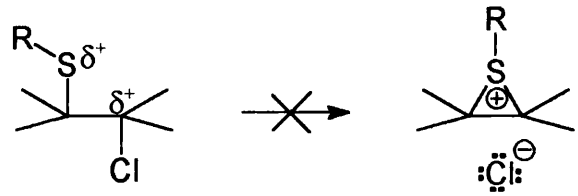
Pathway proposed for methionine-γ-lyase inactivation by 2-amino-4-chloro-5-(chlorodifluoromethylsulfinyl)pentanoic acid

2. Improved yields of the desired regioisomers during synthesis versus the undesirable 1:10 (Markovnikov : anti-Markovnikov ratio described in the synthesis of 2-amino-4-chloro-5-(*p*-nitrophenylsulfinyl)pentanoic acid

In planning the synthesis and composition of this target molecule one not only looked into ways of improving the overall biological activity, but also improving the synthetic yield of the favoured regioisomers and how this might be increased during the addition reaction of a sulfenyl chloride to the protected L-allylglycine.

10.2 Addition reactions of fluorochloromethanesulfenyl chloride to terminal alkenes

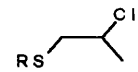
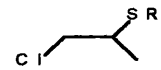
The addition of fluorochloromethanesulfenyl chlorides to alkenes is now well documented (105, 106). K.A. Pretrov and his associates first reported the reactions of Cl_3CSCl and Cl_2FCSCl with cyclohexene, propylene and allyl chloride in 1959 (107). In their paper they reported that these sulfenyl chlorides, in contrast to their hydrocarbon analogues, added to alkenes only with great difficulty. Later in 1985, A. Haas and co-workers (108) were able to show that catalytic amounts of trifluoroacetic acid (TFA) promoted the addition of fluorochloromethanesulfenyl chlorides to terminal alkenes and that an increasing degree of fluorination markedly increases the rate of addition. In the same paper, they were able to demonstrate that fluorochloromethanesulfenyl chloride alkene adducts were sufficiently stable such that they could undergo vacuum distillation. Resistance to isomerisation results from the electron withdrawing effect of the fluorochloromethyl group (as previously discussed for the *p*-nitrophenyl group in the synthesis of 2-amino-4-chloro-5-(*p*-nitrophenylsulfinyl)pentanoic acid). The electron-withdrawing effect of the fluorochloromethyl group renders the sulfur atom electron deficient thus making it inaccessible to attack by the β-carbon. This precludes the formation of the episulfonium ion, which is necessary for isomerisation to occur.



(R = Cl_xF_yC–, where X+Y = 3)

The products afforded by the reaction of fluorochloromethanesulfenyl chlorides with assymetric alkenes were therefore always the kinetic products. The products isolated were usually a mixture of Markovnikov and anti-Markovnikov oriented adducts, with the exact Markovnikov : anti-Markovnikov product ratio greatly depending on the nature of i) the alkene, ii) the sulfenyl chloride and iii) the reaction conditions used. Some examples are given below:

Table 3.

Terminal Alkene	Sulfenyl chloride	Yield	Isomeric Ratio of Adducts Produced	
			Markovnikov 	anti-Markovnikov 
Hex-1-ene	F_3CSCl	90	60	40
Hex-1-ene	$F_2ClCSCl$	96	60	40
Hex-1-ene	FCl_2CSCl	91	70	30
$BrCH{=}CH_2$	F_3CSCl	95	84	14
$BrCH{=}CH_2$	$F_2ClCSCl$	94	87	13
$BrCH{=}CH_2$	FCl_2CSCl	45	87	13

(Reaction conditions varied, with temperatures ranging from -20°C and 80°C, depending on the terminal alkene and sulfenyl chloride used. Full experimental details are cited in reference 108).

On the basis of Haas and co-workers' data (108), it was predicted that the addition of a fluorochloromethanesulfenyl chlorides to a protected L-allylglycine would afford addition products in a greater than 1 : 10 Markovnikov : anti-Markovnikov ratio. (Provided that trifluoroacetic acid is employed as a catalyst and the reaction conditions are favourable).

It was decided to synthesise difluorochloromethanesulfenyl chloride first. Difluorochloromethanesulfenyl chloride is easier to handle than trifluoromethanesulfenyl chloride and more highly fluorinated, and hence more reactive towards the alkene addition, than fluorodichloromethanesulfenyl chloride.

10.3 TFA stable amino acid protecting groups

Having chosen fluorochloromethanesulfenyl chlorides as the group of sulfenyl chlorides one wanted to use, one then faced the issue of identifying TFA stable amino acid protecting groups.

Recall in Walsh and Firestone's synthesis of 2-amino-4-chloro-5-(*p*-nitrophenyl sulfinyl) pentanoic acid (81),TFA had been employed for the simultaneous deprotection of both the Boc- and benzhydryl ester protecting groups as well as for its ability to play an important role in the stabilisation of the final product.

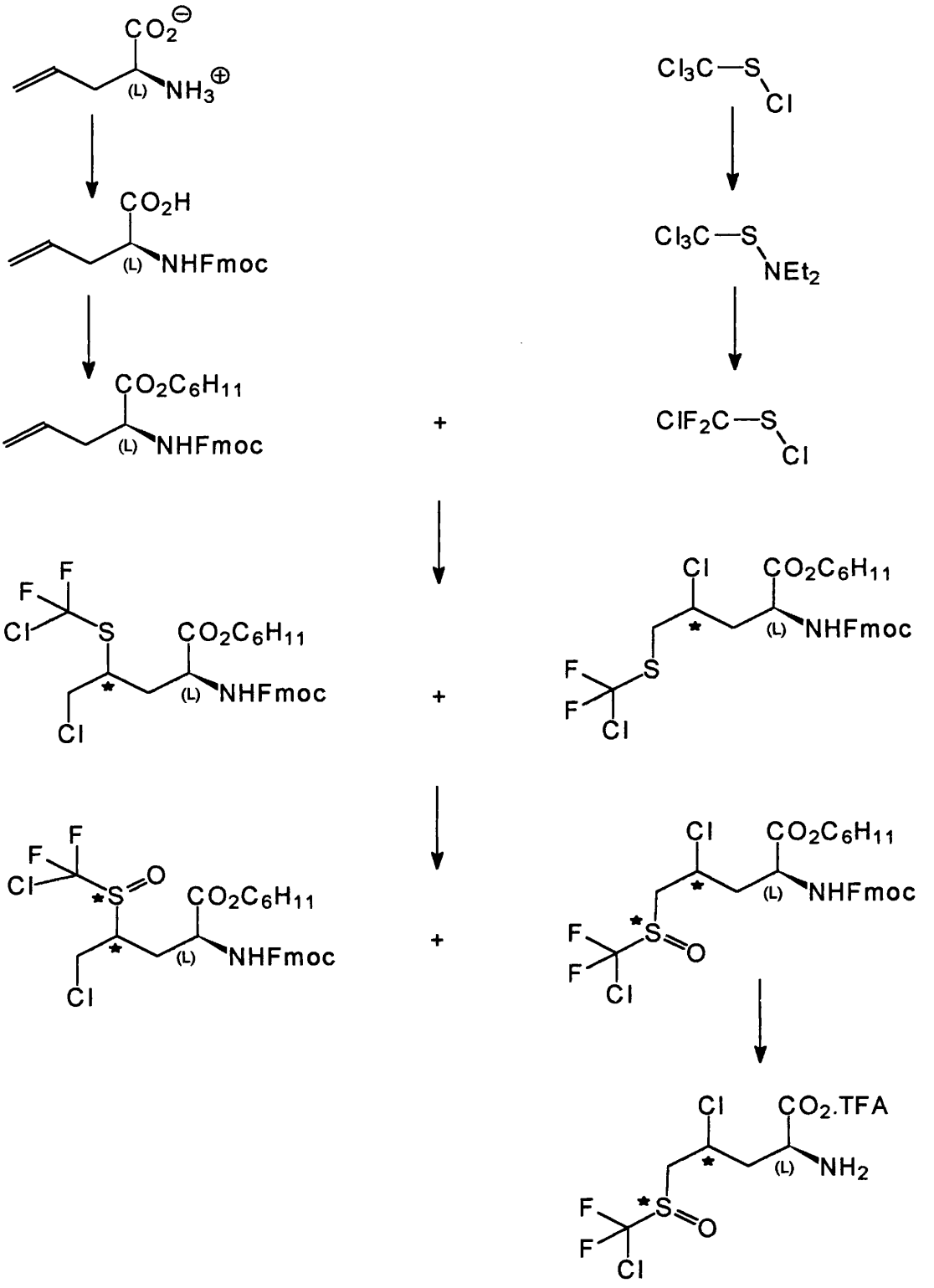
10.3.1 N-Fmoc-protection

The F-moc- protecting group has previously been used by L.A. Caprino and G.Y. Han (109), in the amine protection of the amino acid glycine. It is stable to TFA and reported to be cleaved under mild conditions with an amine base.

10.3.2 Cyclohexyl ester protection

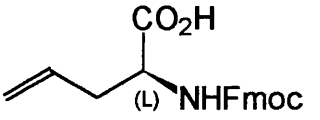
Cyclohexyl esters have been used to protect carboxyl groups in aspartyl peptides during acidic or basic reactions (110). Cycloalkyl esters are stable to amines and TFA but are readily cleaved by methanesulfonic acid or HF (111).

Hence on the basis of both stability and cleavability it was decided that N-Fmoc protection and cyclohexyl ester protection were to be used in this synthesis.



Schematic summary of the synthetic route followed in the preparation of 2-amino-4-chloro-5-(chlorodifluoromethyl sulfinyl)pentanoic acid (★ represents the formation of new chiral centres)

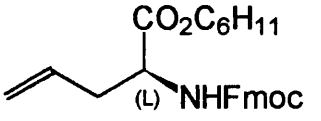
10.4 Synthesis of N-Fmoc-L-allylglycine



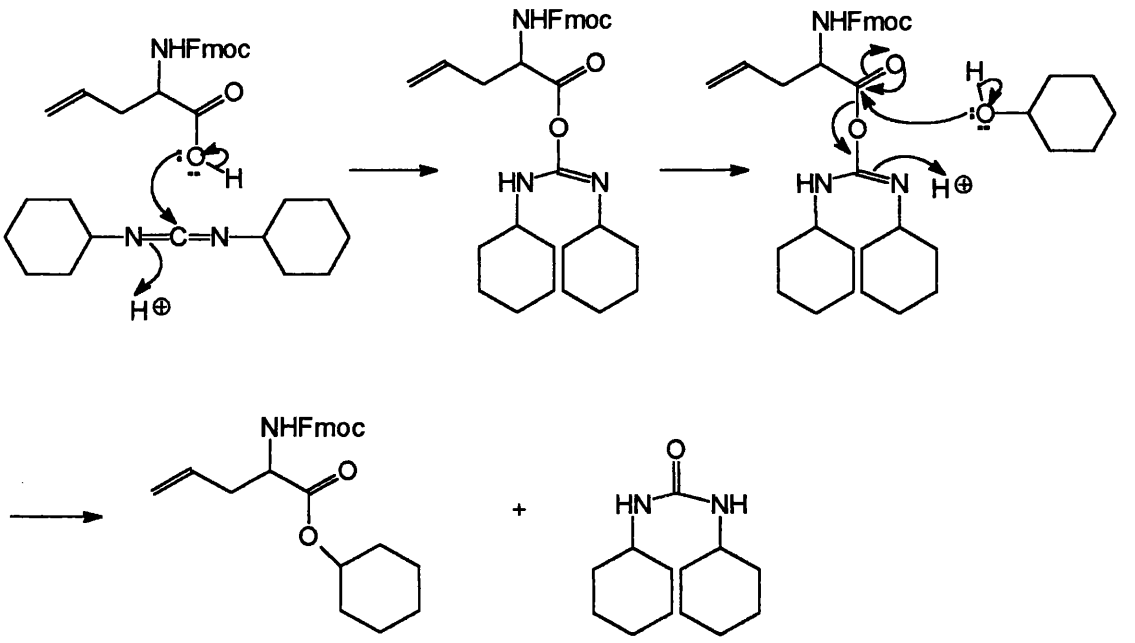
Protection of L-allylglycine using 9-fluorenylmethyl chloroformate (Fmoc-chloride) was successfully achieved following the glycine protection literature procedure of L.A. Caprino and G.Y. Han (109). The insoluble by-products of the reaction, 9-fluorenylmethanol and dibenzofulvene, were easily removed from the reaction mixture by extraction with ether. Following acidification of the aqueous layer the reaction afforded N-Fmoc-L-allylglycine in an 85.6% overall yield from L-allylglycine compared with a literature value of 89%.

IR, ^{1}H NMR, ^{13}C NMR spectra, m/z and micro analysis all support the formation of N-Fmoc-L-allylglycine.

10.5 Synthesis of N-Fmoc-L-allylglycine cyclohexyl ester



N-Fmoc-L-allylglycine cyclohexyl ester was successfully prepared following the general procedure of J. P. Tam and co-workers (111) for carboxylic acid protection. 1,3-Dicyclohexycarbodiimide (DCC) reacts with the Fmoc-L-allylglycine in the presence of a catalytic amount of dimethylaminopyrimidine (DMAP) to form an intermediate product with a good leaving group (urea). The leaving group is displaced by cyclohexanol in a nucleophilic acyl substitution reaction to afford the cyclohexyl ester protected amino acid. The precipitated urea was filtered off and following further purification by column chromatography the reaction afforded an overall yield of 67%.



Mechanism of activation of the $-CO_2H$ group by DCC and the subsequent nucleophilic acyl substitution reaction with cyclohexanol

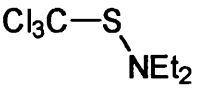
IR, ^1H NMR, ^{13}C NMR spectra, and m/z all support the formation of N-Fmoc-L-allylglycine cyclohexyl ester.

An alternative and reportedly high yielding procedure for the esterification of alcohols with carboxylic acids was published in 1995 by K. Ishihara and associates (112). Their method, employing scandium triflate as a Lewis acid catalyst and *p*-nitrobenzoic acid anhydride, was reported to give excellent esterification yields, often >95%, for a wide range of aliphatic acids and alcohols. On following this general method for the preparation of N-Fmoc-L-allylglycine cyclohexyl ester from N-Fmoc-L-allylglycine and cyclohexanol, one was only able to attain a 62% overall yield. This was a 5% lower yield than that previously obtained by the general method of J.P. Tam and co-workers (111)).

10.6 Difluorochloromethanesulphenyl chloride

During the reaction of trichloromethanesulfenyl chloride with metal fluorides the C–S bond is broken and only fluorochloromethanes are formed (113). However, in 1959 N-N. Yarovenko and co-workers reported that by converting trichloromethane sulfenyl chloride into trichloromethyl-(N-diethyl)-sulfenamide prior to treatment with antimony fluorides, rupture of the C–S bond can be avoided (114). Following fluorination the sulfenamide can then easily be converted back into the sulfenyl chloride on treatment with dry HCl gas.

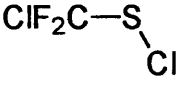
10.6.1 Synthesis of Trichloromethyl-(N-diethyl)-sulfenamide



Following the procedure of H. Lecher and F. Holscheneider (115), trichloromethyl-(N-diethyl)-sulfenamide was prepared in 95% yield from the S_N2 displacement reaction of trichloromethanesulfenyl chloride with diethylamine. This yield compares well with the literature value of 97-98%.

^1H NMR, ^{13}C NMR spectra, and m/z all support the formation of trichloromethyl-(N-diethyl)-sulfenamide.

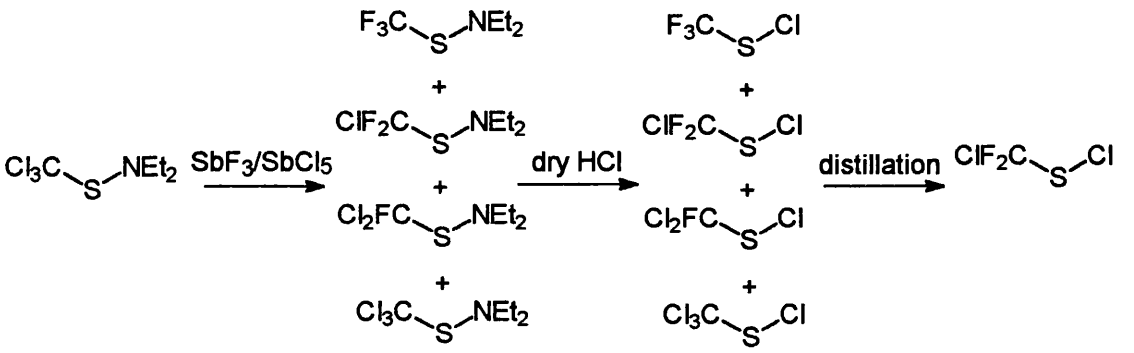
10.6.2 Synthesis of Difluorochloromethanesulphenyl chloride



Following the procedure of N.N. Yarovenko and co-workers (114) difluorochloromethyl-(N-diethyl)-sulfenamide was prepared from the reaction of trichloromethyl-(N-diethyl)-sulfenamide and antimony trifluoride in the presence of small amounts of antimony pentachloride at 67°C.

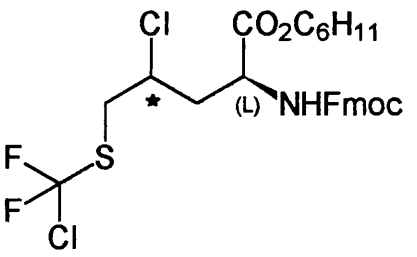
This reaction is very temperature dependent and at temperatures below 65°C practically only fluorochloromethyl-(N-diethyl)-sulfenamide is formed.

Although difluorochloromethyl-(N-diethyl)-sulfenamide is formed in a mixture with fluorodichloro- and trichloromethyl-(N-diethyl)-sulfenamide, due to its instability there was no need to isolate it in a pure form. Instead it was sufficient to separate the mobile liquid from the solid and tarry reaction products and saturate it with dry HCl gas. The difluorochloromethanesulfenyl chloride prepared in this way was then readily separated from trichloro- (b.p. 149°C) and fluorodichloromethanesulfenyl chloride (b.p. 99°C) by kugelrohr distillation in an overall yield of 22% from trichloromethyl-(N-diethyl)-sulfenamide. This compares well with the literature yield of 21%.



The ^{19}F NMR spectrum and b.p. (49°C) support the formation of difluorochloromethanesulfenyl chloride.

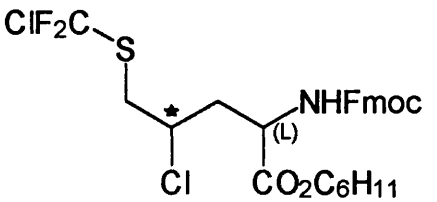
10.7 Synthesis of 2-N-Fmoc-4-chloro-5-(difluorochloromethylsulfenyl) pentanoic acid cyclohexyl ester



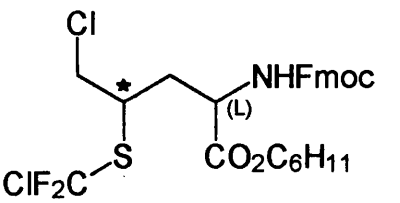
Following the general procedure of A. Haas and associate (108), 2-N-Fmoc-4-chloro-5-(difluorochloromethylsulfenyl)pentanoic acid cyclohexyl ester was prepared along with its regioisomer 2-N-Fmoc-5-chloro-4-(difluorochloromethyl sulfenyl)pentanoic acid cyclohexyl ester in an overall 19.1% yield. This was a disappointing yield when compared with the literature examples for the addition of difluorochloromethanesulfenyl chloride to hex-1-ene and bromoethene which afforded >90% yields (see table 3.). However this may have been due to the bulky F-moc- and cyclohexyl protecting groups retarding the approach of the sulfenyl chloride to the double bond resulting in a lower yield. This rationale is in line with the literature yields obtained in the synthesis of 2-N-Boc-4(5)-chloro-5(4)-(*p*-nitrophenylsulfinyl)pentanoic acid benzhydryl ester which were 9-10% (81).

Alternatively, the reaction conditions employed may not have been at an optimum for either the alkene or sulfenyl chloride used. However there was not time to explore these variables.

As predicted earlier on the basis of Haas and co-workers data (108), the addition of difluorochloromethanesulfenyl chloride to the protected L-allylglycine did afford addition products in a greater than 1 : 10 Markovnikov : anti-Markovnikov ratio. Integration of the ^{1}H NMR signals of the unpurified product mixture indicated the ratio of regioisomers **B** : **C** (Markovnikov : anti-Markovnikov) was approximately 2 : 3.



B



C

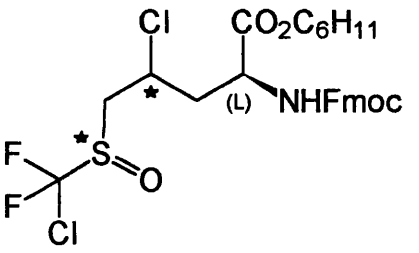
Markovnikov (2 : 3 product ratio) anti-Markovnikov

(* indicates the new chiral centre)

^{1}H NMR, ^{13}C NMR and ^{19}F NMR spectra all support the formation of 2-N-Fmoc-4(5)-chloro-5(4)-(chlorodifluoromethylsulfenyl)pentanoic acid cyclohexyl ester

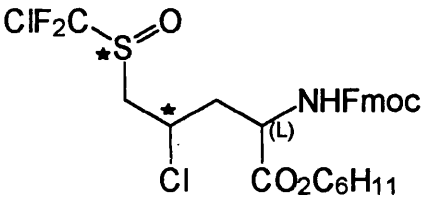
In particular, the proximity of the fluorine atoms to the new chiral centre in both the Markovnikov product **B** and anti-Markovnikov product **C** is reflected in the ^{13}C NMR and ^{19}F NMR spectra. The 2 fluorine atoms in the Markovnikov product **B** are magnetically equivalent and this is supported by the observation of a triplet (132.2ppm, J = 337.2Hz), in the ^{13}C NMR spectrum and a singlet (-25.81ppm) in the ^{19}F NMR spectrum. In the anti-Markovnikov product **C** the fluorine atoms are in close proximity to the new chiral centre and are magnetically non-equivalent. The non-equivalent fluorine atoms can be seen as 2 doublets (128.0 and 134.1ppm, J = 315Hz) in the ^{13}C NMR spectrum and 2 multiplet signals in the ^{19}F NMR spectrum (-25.30 and -26.88 ppm).

10.8 Synthesis of 2-N-Fmoc-4-chloro-5-(difluorochloromethylsulfinyl) pentanoic acid cyclohexyl ester

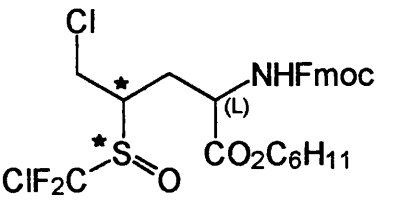


Following the literature procedure described by C. Walsh & R. A. Firestone, (81), 2-N-Fmoc-4-chloro-5-(difluorochloromethylsulfinyl)pentanoic acid cyclohexyl ester was successfully prepared from the oxidation of the mixed regioisomers 2-N-Fmoc-4(5)-chloro-5(4)-(difluorochloromethylsulfenyl) pentanoic acid cyclohexyl ester with 55% mCPBA in DCM at 0°C. Separation of the desired product **D** from the undesired product **E** was achieved by repeated flash chromatography and afforded an overall isolated yield of 21.2% from the protected L-allylglycine (53% based on the 2 : 3 ratio of **B** : **C**).

The isolated compound **D** exists as 4 diastereoisomers, with the creation of the new chiral centre being brought about by the oxidation of sulfide to sulfoxide.



D

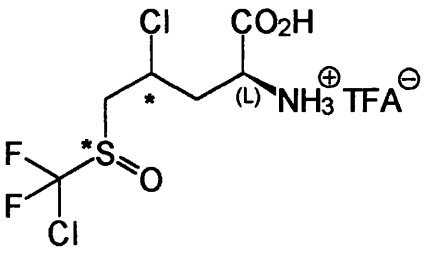


E

The ^{13}C NMR and ^{19}F NMR spectra support the formation of 2-N-Fmoc-4-chloro-5-(chlorodifluoromethylsulfinyl)pentanoic acid cyclohexyl ester **D** as diastereoisomers.

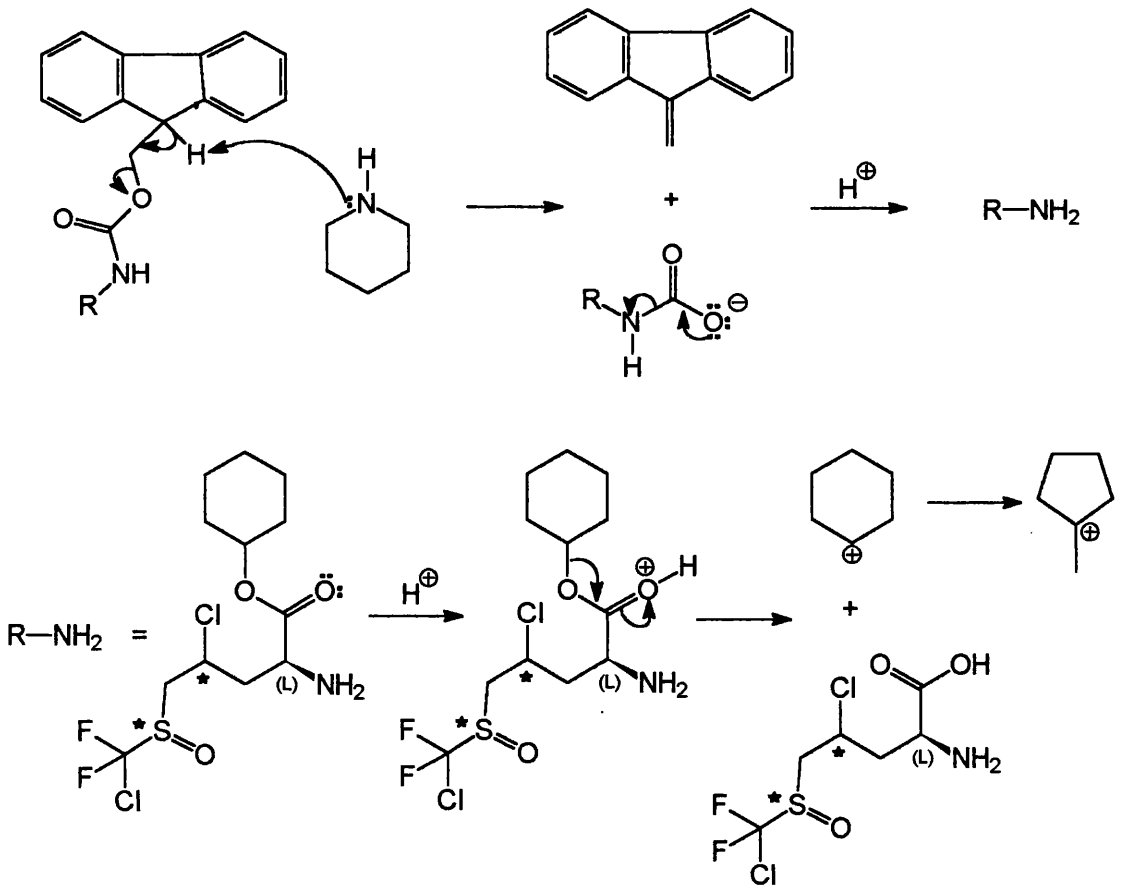
In particular, in the ^{19}F NMR spectrum a triplet at -60.2 ppm for one diastereoisomer (equivalent F's being split by 2 equivalent δH's) and a doublet of doublets at -54.6 ppm for a second diastereoisomer (equivalent F's being split by 2 non-equivalent δH's) were observed. The 2 remaining diastereoisomers appear as overlapping multiplet signals at -58.6 ppm and -60.2 ppm.

10.9 Synthesis of 2-Amino-4-chloro-5-(chlorodifluoromethylsulfinyl)pentanoic acid.TFA salt



It was intended to sequentially deprotect the amine group followed by the carboxyl group of 2-N-Fmoc-4-chloro-5-(chlorodifluoromethylsulfinyl) pentanoic acid cyclohexyl ester, while minimising the opportunity for intramolecular displacement reactions, to afford the free amino acid as the TFA salt. In the literature the Fmoc- protecting group is cleavable under mild conditions with an

amine base (109). 20% Piperidine in DMF is reported to cleave the amine group in a matter of seconds at room temperature (the actual time required depends on the structure of the Fmoc- amine derivative). The cyclohexyl protecting group on the carboxyl is stable to base but is reported to be cleaved by methanesulfonic acid in 1 h at 0°C (111). Following these two general literature procedure for Fmoc- deprotection and cyclohexyl deprotection, one proceeded to attempt a sequential deprotection process. One added 20% piperidine in DMF and stirred the reaction mixture at room temperature for 1 min. The reaction flask was then cooled to 0°C and methanesulfonic acid and 2 drops of TFA added. This was then stirred at 0°C for 1h. The reaction was monitored by TLC / ninhydrin spray but unfortunately, even after a second attempt, no free amino acid was observed. Starting material D amounting to 55mgs was the only product recovered.



Anticipated reaction mechanism for the sequential deprotection of 2-N-Fmoc-4-chloro-5-(difluorochloromethylsulfinyl)pentanoic acid cyclohexyl ester

Following Fmoc- deprotection, polymerised dibenzofulvene should have been a byproduct of the reaction and in theory this insoluble polymer would have precipitated out of solution. This was not observed. The cyclohexyl carbenium ion formed during the deprotection of the carboxyl group would have become inactivated via rearrangement to a cyclopentyl carbenium ion. Together this sequential deprotection process was intended to afford the free amino acid protected as the TFA salt.

Unfortunately due to time constraints it was not possible to ascertain whether it was the reaction conditions employed or the reagents used that resulted in the deprotection sequence not proceeding as expected.

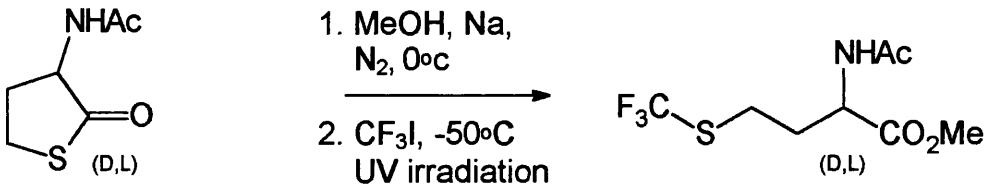
Having run out of time no further deprotection procedures were attempted. However, use of a less bulky amine, such as ammonia, might have proved more effective in removing the Fmoc- group in this system. An alternative to using methanesulfonic acid for the removal of the cyclohexyl group would have been to try HF gas.

EXPERIMENTAL

General Methods:

Melting points were determined on a Kofler hot stage melting point apparatus and are uncorrected. Short path distillations were carried out on a Büchi GKR-50 Kugelrohr. Recorded boiling ranges refer only to the indicated air bath temperature. ^{1}H NMR spectra were recorded on a Bruker AM200SY or a Bruker WP200SY spectrometer both operating at 200MHz or on a Perkin Elmer R32 spectrometer operating at 90MHz. ^{13}C NMR spectra were recorded on a Bruker AM200SY spectrometer operating at 50 MHz. ^{19}F NMR Spectra were recorded on a Bruker AM200SY spectrometer operating at 188 MHz. Chemical shifts in the ^{1}H, ^{13}C and ^{19}F NMR spectra are reported in parts per million (δ) relative to residual proton shifts in deuteriochloroform at 7.24 ppm for the ^{1}H and ^{19}F NMR and the central signal at 77.0 ppm in the ^{13}C NMR spectrum. Coupling constants (*J*) are quoted in Hertz. The multiplicities stated in the ^{13}C NMR spectra were determined by the use of DEPT spectra with pulse angles ϕ = 90° and 135°. Data are reported using the following convention: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b= broadened. Infrared spectra were recorded on a Perkin Elmer 983 or a Perkin Elmer P-1000 spectrometer. Mass spectra were obtained using a VG/Kratos MS12 spectrometer or a VG/Kratos MS90S spectormeter for high resolution work. The U.V. light source was supplied by a Hannovia Medium Pressure Mercury Lamp, 125 watts, unit number 8025. Separation of compounds was carried out by dry flash chromatography, under reduced pressure using Merck Kieselgel 60 or alternatively by continuous reverse phase chromatography under positive pressure using Fluka reverse phase silica gel 100 C_{18} unless otherwise stated.

N-Acetyl-D,L-trifluoromethionine methyl ester



adapted procedure of: M.E. Houston and J.F. Honek, *J. Chem. Soc., Chem. Comm.*, 1989, 761

Analar MeOH (30 ml) in a Pyrex 3 neck round bottomed flask (50ml) fitted with a dry ice/acetone condenser and a gas inlet tube was cooled to 0^0C in an ice bath. Sodium (0.12g, 5.2mmol) was added portionwise under N_2. N-Acetyl-homocysteine thiolactone (0.796 g, 5.0 mmol) was added all at once and the solution stirred for 30min. The solution was then cooled to –50°C in a dry ice/acetone bath. Trifluoromethyl iodide (2.94g, 15mmol) was measured out on a calibrated vacuum line and trapped in a purpose built fixed volume Pyrex flask (see diagram). The trifluoromethyl iodide was then condensed into the reaction vessel and the mixture stirred and irradiated with a long wave U.V. lamp for 2h at -50°C (in the literature procedure the reaction temperature was kept constant at 0°C for the duration of the expt.). The UV lamp was then removed and the solvent evaporated *in vacuo*. The residue was taken up in EtOAc (50ml) and washed with 5% $NaHCO_3$ solution (25ml) and brine (25ml), dried over Na_2SO_4 and filtered. The filtrate was evaporated *in vacuo* and the resulting yellow oil chromatographed on silica using EtOAc/hexane (3:2) giving 829mg (64%) of the title product as a clear oil.

TLC (3:2, EtOAc : Hexane)

starting material	R_f = 0.39
sodium salt	R_f = 0.00
Product	R_f = 0.57

ν_{max}: (KBr disc) cm^{-1}

1679, 1739, 3425

^1H NMR ($CDCl_3$) δ

2.03 (3H, s, NHCOC<u>H</u>$_3$), 1.97 - 2.37 (2H, m, $CH_2\beta$), 2.95

(2H, t, J = 7.6 Hz, $CH_2\gamma$), 3.76 (3H, s, $CO_2C\underline{H}_3$),

4.71 (1H, t of d, J = 8.1 Hz (t), 5.0 Hz (d), $CH\alpha$),

7.17 (1H, d, J = 7.5 Hz, N<u>H</u>Ac),

^{13}C NMR ($CDCl_3$) δ

22.6 (NHCO<u>C</u>H$_3$), 25.9 ($CH_2\beta$), 32.5 ($CH_2\gamma$), 51.0, (OCH_3), 52.4 ($CH\alpha$),

130.8 (q, J_{CF} = 306.0 Hz, CF_3), 170.7 (NH<u>C</u>OCH$_3$), 171.9 (<u>C</u>O$_2$CH$_3$)

^{19}F NMR ($CDCl_3$) δ

–41.9 (CF_3)

m/z

found 259.0477 (M^+), 158.0816 ($M^+ - CF_3S$)

requires 259.0490 (M^+), 158.0817 ($M^+ - CF_3S$)

Microanalysis (%)

found C 37.24, H 4.73, N 5.39

requires C 37.06, H 4.67, N 5.41

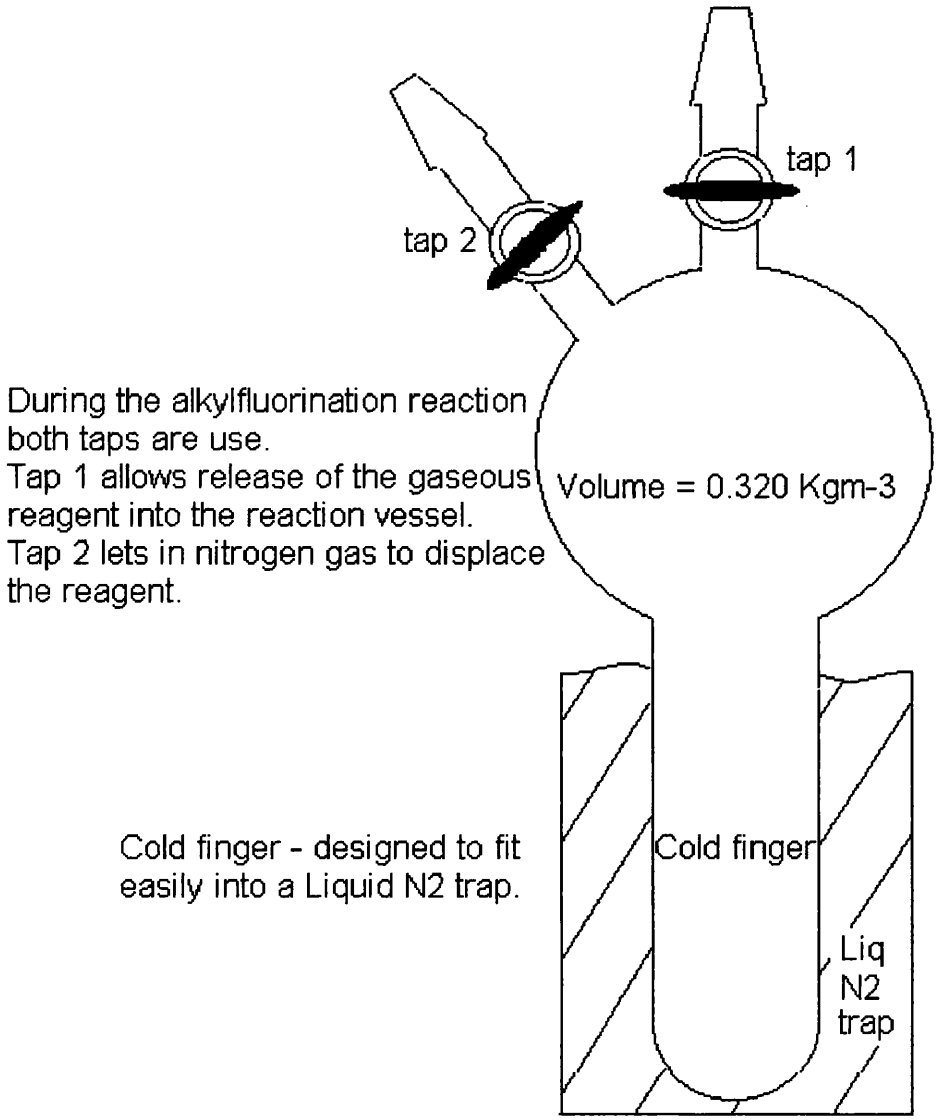
To measure out a known amount of reagent gas:

i) The purpose built ampoule is fitted, via tap 1, and opened to the vacuum line - this allows air inside the ampoule to be evacuated.

ii) Tap 1. is then closed and a known amount of reagent gas is measured out into the vacuum line (the supply cylinder is also attached to the vacuum line).

iii) Tap 1. is then re-opened and, with the aid of a Liq.N_2 trap, the reagent gas condenses into the ampoule.

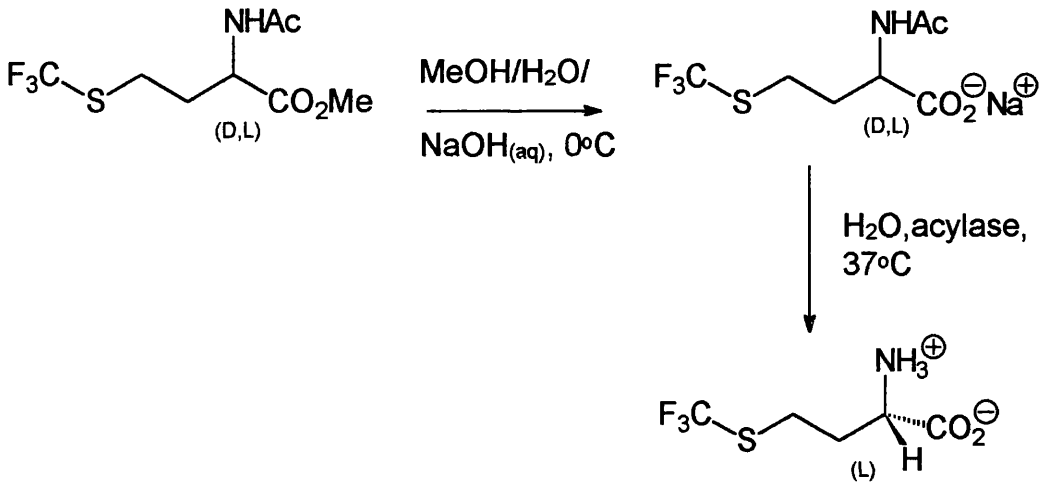
iv) Once all the gas has condensed into the ampoule tap 1 is closed and the reagent gas is ready for use.



purpose built (volume) vacuum line compatible pyrex ampoule

(taps 1. and 2. are made from PTFE)

L-Trifluoromethionine



procedure: M.E. Houston and J.F. Honek, *J. Chem. Soc., Chem. Comm.*, 1989, 761

A single neck round bottomed flask (50ml) charged with N-acetyl-trifluoromethionine methyl ester (700mg, 2.70mmol) and MeOH/H_2O (1:1, 25ml) was cooled to 0°C in an ice bath. To this was added an aqueous solution of NaOH (0.12g, 3.0mmol) and the mixture was stirred for 50min (the reaction was monitored by TLC to assure completion). The solvent was evaporated *in vacuo* giving the sodium salt. The sodium salt was dissolved in degassed distilled H_2O (150ml), transferred to a conical flask and the solution adjusted to pH 7.5 with 0.1M HCl. Porcine kidney acylase (Grade I, 1 mg, 1,430 units, supplied by Sigma Product No. A3010) was added, the flask stoppered and the aqueous solution gently agitated overnight at 37°C in a water bath. The solution was adjusted to pH 5 with 6M HCl and activated charcoal (0.5g) added. The mixture was heated with stirring to 60°C for 10min and filtered. The filtrate was adjusted to pH 1.5 with 6 M HCl and extracted with EtOAc (3 × 15ml). The aqueous layer was applied to a Dowex-50W RSO_3H column and eluted with H_2O until the eluate was the same pH as the distilled H_2O. The

column was then eluted with 1.0 M NH_4OH and the eluate collected and evaporated *in vacuo.* The product was dissolved in distilled H_2O and applied to a reverse phase C-18 silica column and eluted with H_2O. Fractions were monitored by TLC using ninhydrin spray to visualise free amino acid. Amino acid containing fractions were combined and evaporated *in vacuo* giving 207 mg (37.7%, 75.4% based on D,L mixture) of the amino acid as a white solid. m.p. 229 - 230°C.

TLC (50% MeOH, 15% H_20, 35% Et_20)
Product R_f = 0.62

^{1}H NMR (D_2O) δ
2.02 - 2.14 (m, 2H, $CH_2\beta$), 2.92 (t, 2H, J = 7.6 Hz, $CH_2\gamma$),
3.67 (t, 1H, J = 6.4 Hz, CHα),

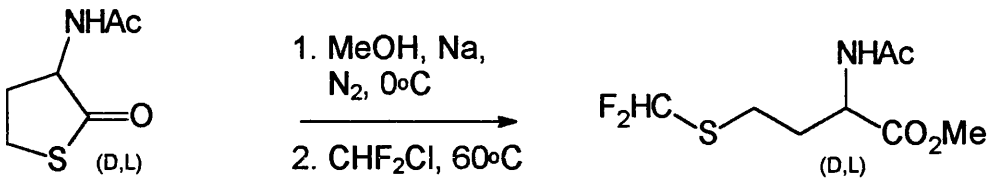
^{13}C NMR (D_2O) δ
26.2 ($CH_2\beta$), 31.9 ($CH_2\gamma$), 54.2 (CHα), 131.7 (q, J_{CF} = 306.0 Hz, CF_3),
174.4 (CO_2H)

^{19}F NMR (D_2O) δ
–41.6 (CF_3)

$[\alpha]_D^{22}$ +16.6 ± 0.5 (5M HCl)
literature value $[\alpha]_D^{25}$ +52.3 ± 0.5 (4M HCl)

m/z
found 203.0230 (M^+), 158.0235 ($M^+ - CO_2H$)
requires 203.0228 (M^+), 158.0251 ($M^+ - CO_2H$)

N-Acetyl-D,L-difluoromethionine methyl ester



adapted procedure of: M.E. Houston and J.F. Honek, *J. Chem. Soc., Chem. Comm.*, 1989, 761

Analar MeOH (40ml) in a 2 neck round bottomed flask (50ml) was cooled to 0 °C in an ice bath. Sodium (0.19g, 8.3mmol) was added portionwise under N_2. N-Acetylhomocysteine thiolactone (1.25 g, 15.8 mmol) was added all at once and the solution stirred for 30min. The mixture and a magnetic stirrer were then transferred to a pressure bottle (100ml). The pressure bottle was attached to a vacuum line and the air carefully evacuated. *Freon 22* (chlorodifluoromethane) (1.38g, 16.0mmol) was measured into the calibrated vacuum line before being condensed directly into the pressure tube. Liquid N_2 was used as the coolant. The tube was sealed and the mixture heated to 60°C with stirring for 10h (in the literature procedure an excess of *freon 22* was bubbled through the reaction mixture over the course of 10h at atmospheric pressure). The reaction mixture was then evaporated *in vacuo* and the residue taken up in EtOAc (50ml), washed with 5% $NaHCO_3$ aqueous solution (25ml) and brine (25ml), dried over Na_2SO_4 and filtered. The filtrate was evaporated *in vacuo* and the resulting yellow oil chromatographed on silica using EtOAc/Petroleum ether (1:2) giving 1.04g (55%) of the desired product as a clear oil.

TLC (3:1, EtOAc : Pet ether)

sodium salt $R_f = 0.00$

Product $R_f = 0.31$

ν_{max}: (KBr disc) cm^{-1}

1674, 1738, 3427

^1H NMR ($CDCl_3$) δ

2.04 (3H, s, NHAc), 1.98 - 2.30 (2H, m, $CH_2\beta$), 2.86 (2H, t, J = 7.7 Hz, $CH_2\gamma$), 3.75 (3H, s, CO_2CH_3), 4.67 (1H, t of d, J = 8.1 Hz (t), 4.8 Hz (d), $CH\alpha$), 6.86 (1H, t, J_{HF} = 56.1 Hz, CF_2H), 7.32 (1H, d, J = 7.9 Hz, NHAc)

^{13}C NMR ($CDCl_3$) δ

22.8 ($NHCOCH_3$), 23.2 ($CH_2\gamma$), 33.1 ($CH_2\beta$), 51.2 (OCH_3), 52.5 ($CH\alpha$), 120.6 (t, J_{CF} = 272.2 Hz, CF_2H), 170.7 ($NHCOCH_3$), 172.2 (CO_2CH_3)

^{19}F NMR ($CDCl_3$) δ

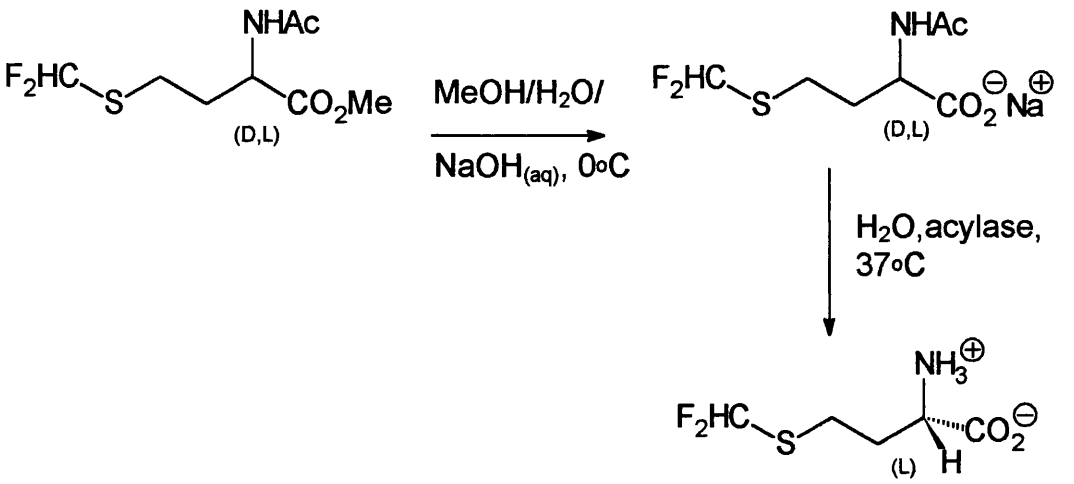
–93.5 (d, J_{HF} = 54.6 Hz, CF_2H)

m/z

found 241.0246 (M^+)

requires 241.0586 (M^+)

L-Difluoromethionine



procedure: M.E. Houston and J.F. Honek, *J. Chem. Soc., Chem. Comm.*, 1989, 761

A single neck round bottomed flask (50ml) charged with N-acetyl-difluoromethionine methyl ester (1.0g, 4.15mmol) was dissolved in MeOH/H_2O (1:1, 25ml) and cooled to 0°C in an ice bath. To this was added an aqueous solution of NaOH (0.18g, 4.5mmol) and the mixture was stirred for 50min (the reaction was monitored by TLC to assure completion). The solvent was evaporated *in vacuo* giving the sodium salt. The sodium salt was dissolved in degassed distilled H_2O (200ml) and the solution adjusted to pH 7.5 with 0.1M HCl. Porcine Kidney acylase (1.7mg, 2,431units, supplied by Sigma Product No. A3010) was added and the solution gently agitated overnight at 37°C in a water bath. The amino acid was purified as previously described giving 315mg (41.1%, 82.4% based on D,L mixture) of the title product as a white solid. m.p. 200°C (literature value 199 - 201°C).

^{1}H NMR (D_2O) δ

2.03 (2H, m, $CH_2\beta$), 2.75 (2H, t, J = 7.6 Hz, $CH_2\gamma$), 3.63 (1H, t, J = 6.0 Hz, $CH\alpha$), 6.88 (1H, t, J_{HF} = 51.8 Hz, CF_2H)

^{13}C NMR (D_2O) δ

22.5 ($CH_2\gamma$), 31.4 ($CH_2\beta$), 53.3 ($CH\alpha$),120.9 (t, J_{CF} = 276.7 Hz, CF_2H), 174.2 (CO_2H)

^{19}F NMR (D_2O) δ

–96.1 (d, J_{HF} = 56.5 Hz, CF_2H)

$[\alpha]_D^{22}$ +6.8 $\pm$ 0.5 (5 M HCl)

no literature value given

m/z

found 140.0334 ($M^+ - CO_2H$), 74.0241 ($M^+ - CH_2CH_2SCHF_2$)

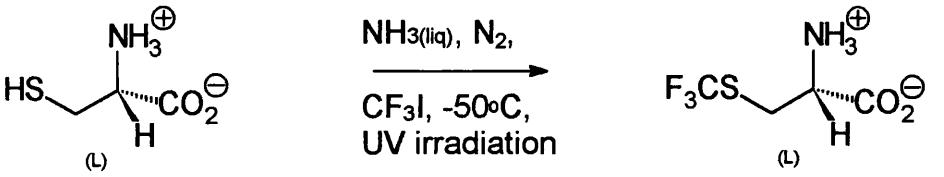
requires 140.0346 ($M^+ - CO_2H$), 74.0242 ($M^+ - CH_2CH_2SCHF_2$)

microanalysis (%)

found C 32.57, H 4.79, N 7.74

requires C 32.43, H 4.90, N 7.57

L-Trifluoromethyl cysteine



procedure: V. Soloshonok, V. Kukhar, Y. Pustovit and V. Nazaretian
Synlett., 1992, 657

Liquid NH_3 (50ml) in a Pyrex 3 neck round bottomed flask (100ml) fitted with a dry ice/acetone condenser was cooled to -50^0C in a dry ice/acetone bath. L-Cysteine (0.700g, 5.78mmol) was added all at once and the solution stirred under N_2. Trifluoromethyl iodide (2.94g, 15.0mmol) was measured out using the purpose built flask and introduced to the reaction vessel as previously described. The solution was irradiated with a long wave UV lamp for 1.5h. The UV lamp was removed and the solvent evaporated *in vacuo.* The solid white residue was taken up in MeOH (30ml) and filtered of insoluble material. The filtrate was evaporated *in vacuo* and the residue taken up in distilled H_2O (200ml). The solution was adjusted to pH 1.5 with 6M HCl and applied to a Dowex-50W RSO_3H column. The column was eluted with H_2O until the eluate was the same pH as the distilled H_2O. The column was then eluted with 1.0M NH_4OH and the eluate collected and evaporated *in vacuo.* The product was dissolved in distilled H_2O and applied to a reverse phase C-18 column and eluted with H_2O. Fractions were monitored by TLC using ninhydrin spray to visualise free amino acid. Fractions were combined and evaporated *in vacuo* giving 790mg (73.2%) of free amino acid as a white solid. m.p. 229 - 231°C (literature value 227 - 230°C).

TLC (50% MeOH, 15% H_2O, 35% Et_2O)
Starting material $R_f = 0.28$

Product $R_f = 0.70$

^{1}H NMR (D_2O) δ

3.30 (CH_2), 3.86 (CH), (3H, AA'B system, $J_{AA'} = 15.9$ Hz, $J_{AB} + J_{A'B} = 12.0$ Hz)

(AA' and B represents CH_2 and CH protons respectively)

^{13}C NMR (D_2O) δ

30.7 (CH_2), 75.4 (CH), 131.1 (q, $J_{CF} = 306.0$ Hz, CF_3), 172.3 (CO_2H)

^{19}F NMR (D_2O) δ

–41.6 (CF_3)

$[\alpha]_D^{22}$ +18.2 ± 0.5 (5M HCl)

literature value $[\alpha]_D^{25}$ +24.1 ± 0.5 (4M HCl)

m/z

found 189.0061 (M^+), 144.0114 ($M^+ - CO_2H$), 74.0244 ($M^+ - F_3CSCH_2$)

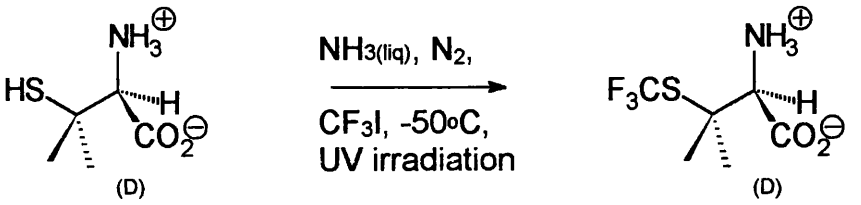
requires 189.0071 (M^+), 144.0095 ($M^+ - CO_2H$), 74.0242 ($M^+ - F_3CSCH_2$)

microanalysis (%)

found C 25.50, H 3.19, N 7.31

requires C 25.40, H 3.20, N 7.41

D-Trifluoromethyl penicillamine



procedure: V. Soloshonok, V. Kukhar, Y. Pustovit and V. Nazaretian

Synlett., 1992, 657

Trifluoromethylation of D-penicillamine was executed by the same method described for L-cysteine. The quantities used were: NH_3 (50ml), D-penicillamine (0.777g, 5.2mmol), and trifluoromethyl iodide (2.94g, 15.0mmol). This gave 960 mg (65.7%) of the free amino acid as a white solid. m.p. 209 - 210°C

TLC (50% MeOH, 15% H_2O, 35% Et_2O)

Starting material $R_f = 0.47$

Product $R_f = 0.64$

^{1}H NMR (D_2O) δ

1.61,1.27 (s,s, $C(CH_3)_2$), 3.75 (s,CHα)

^{13}C NMR (D_2O) δ

28.6, 23.9 ($(CH_3)_2$), 52.1 ($\underline{C}(CH_3)_2$), 63.1 (CHα), 130.8 (q, J_{CF} = 305.0 Hz, CF_3), 171.0 (CO_2H)

^{19}F NMR (D_2O) δ

– 36.5 (CF_3)

$[\alpha]_D^{22}$ –0.79 ± 0.5 (5M HCl)

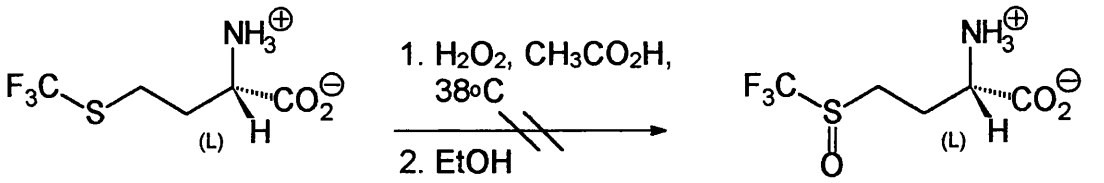
literature value $[\alpha]_D^{25}$ –4.1 ± 0.5 (6M HCl)

m/z

found 172.0410 ($M^+ - CO_2H$), 71.0734 ($M^+ - F_3CS$, CO_2H)

requires 172.0408 ($M^+ - CO_2H$), 71.0735 ($M^+ - F_3CS$, CO_2H)

L-Trifluoromethionine sulfoxide

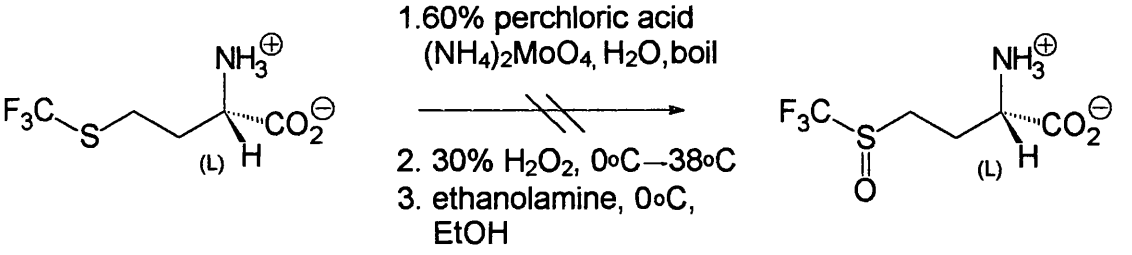


product has never successfully been prepared

general procedure: A. Lepp and M.S. Dunn, *Biochemical Preparations,* 1955, Vol 4, 80

Glacial acetic acid (10ml) and trifluoromethionine (500mg, 2.46mmol) were mixed in a conical flask to form a slurry. Slowly with stirring 30% H_2O_2 (0.33ml, 2.81mmol) was added dropwise to the flask. Once addition was complete the mixture was stirred for a further 5min at room temperature. The flask was covered with an inverted beaker and stored in an oven at 38°C overnight. The reaction mixture was removed from the oven allowed to cool to room temperature and EtOH (5ml) was added with shaking. The flask was placed at the back of the fume hood (cold area) during the interval of precipitation (24h). The precipitate was filtered, washed with EtOH (10ml) and dried. Only starting material was recovered.

L-Trifluoromethionine sulfoxide

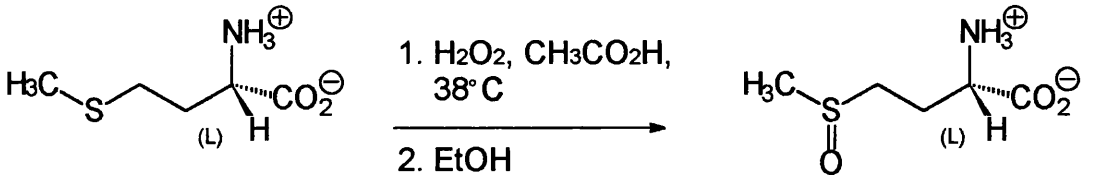


product has never successfully been prepared

general procedure: A. Lepp and M.S. Dunn, *Biochemical Preparations,* 1955, Vol 4, 81

To a round bottomed flask (5ml), 60% perchloric acid (0.27ml, 2.43mmol)), $(NH_4)_2MoO_4$ (34mg, 0.17mmol) and H_2O (1ml) were added. The mixture was boiled for 5min and hot filtered of any undissolved material. The hot filtrate and trifluoromethionine (160mg, 0.7 mmol) were mixed in a conical flask to form a slurry and cooled in an ice bath. Slowly with stirring 30% H_2O_2 (0.30ml, 2.53mmol) was added dropwise to the flask. Once addition was complete the mixture was stirred for a further 5min at room temperature. The flask was covered with an inverted beaker and stored in an oven at 38°C over night. The mixture was neutralised to pH 5 with ethanolamine and cooled in an ice bath. The flask was placed at the back of the fume hood and EtOH (5ml) added. After 24h the precipitate was filtered and washed with EtOH (10ml) and dried. Only starting material was recovered.

L-Methionine sulfoxide



procedure: A. Lepp and M.S. Dunn, *Biochemical Preparations,* 1955, Vol 4, 80

Glacial acetic acid (25ml) and L-methionine (5.0g, 24.6mmol) were mixed in a conical flask to form a slurry. Slowly with stirring 30% H_2O_2 (4.50ml, 38.3mmol) was added dropwise to the flask. Once addition was complete the mixture was stirred for a further 5min at room temperature. The flask was covered with an inverted beaker and stored in an oven at 38°C overnight. The reaction mixture was removed from the oven allowed to cool to room temperature and EtOH (15ml) was added with shaking. The flask was placed at the back of the fume hood (cold area) during the interval of precipitation (24h). The precipitate was filtered, washed with EtOH (50ml) and dried to yield 3.02g (58.0%) of the title product methionine sulfoxide with a cabbage like odour. m.p. 230°C (literature value 232°C).

Starting material (0.87g) was also recovered.

TLC (50% MeOH, 15%H_2O, 35% Et_2O)

starting material $R_f = 0.71$

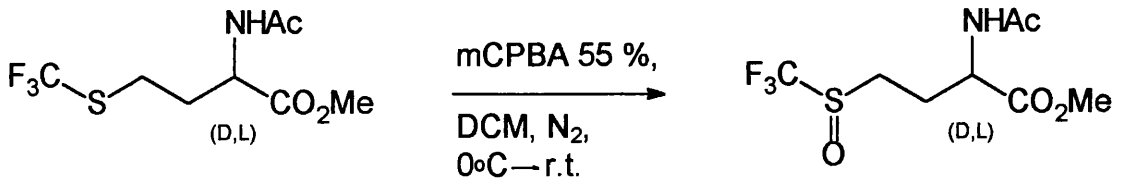
product $R_f = 0.38$

m/z

found 165.0486

requires 165.2170

N-Acetyl-D,L-trifluoromethionine sulfoxide methyl ester



product has never before been prepared

procedure: M. Johnston, R. Raines, C. Walsh and R. A. Firestone, *J. Am. Chem. Soc.*, 1980, **102**, 4241

A flame dried round bottomed flask (25ml) equipped with side arm and septum cap, under N_2, was charged with trifluoromethionine methyl ester (1.00g, 4.93mmol) in distilled DCM (25ml) and stirred at 0°C in an ice bath. A solution of 55% mCPBA (1.54g, 4.93mmol) in distilled DCM (25ml), with added activated 4Å molecular sieves (2.0g) to reduce the overall water content, was added to the reaction flask via syringe over 1h. After 30min at room temperature the solution was washed with $NaHCO_3$ solution (3 × 50ml), dried over Na_2SO_4, and filtered. The filtrate was evaporated in *vacuo* to give a yellow oil. The oil was applied to a silica column and eluted with EtOAc to give 1.04g (91.6%) of the title product as a clear oil.

TLC (EtOAc)

starting material $R_f = 0.70$

product $R_f = 0.51$

m/z

found 232.0228 ($M^+ - COCH_3$)

requires 232.0255 ($M^+ - COCH_3$)

^{1}H NMR ($CDCl_3$) δ

1.97 (3H, s, NHCOC<u>H</u>$_3$), 2.16 (1H, m, $CH_2\beta$), 2.35 (1H, m, $CH_2\beta$),

3.01 (2H, m, $CH_2\gamma$), 3.70 (3H, s, $CO_2C\underline{H}_3$), 4.67 (1H, m, CHα),

7.12 (1H, d, J = 8.0 Hz, N<u>H</u>Ac),

^{13}C NMR ($CDCl_3$) δ

22.6 (NHCO<u>C</u>H_3), 24.2, 24.5 ($CH_2\beta$), 44.7 ($CH_2\gamma$), 50.7, 50.9 (OCH_3),

52.6 (CHα), 125.1 (q, J_{CF} = 331.5 Hz, CF_3), 170.8 (NH<u>C</u>OCH_3), 171.3 (<u>C</u>O_2CH_3)

^{19}F NMR ($CDCl_3$) δ

–73.61, –73.64 (CF_3)

m/z

found 232.0228 ($M^+ - COCH_3$)

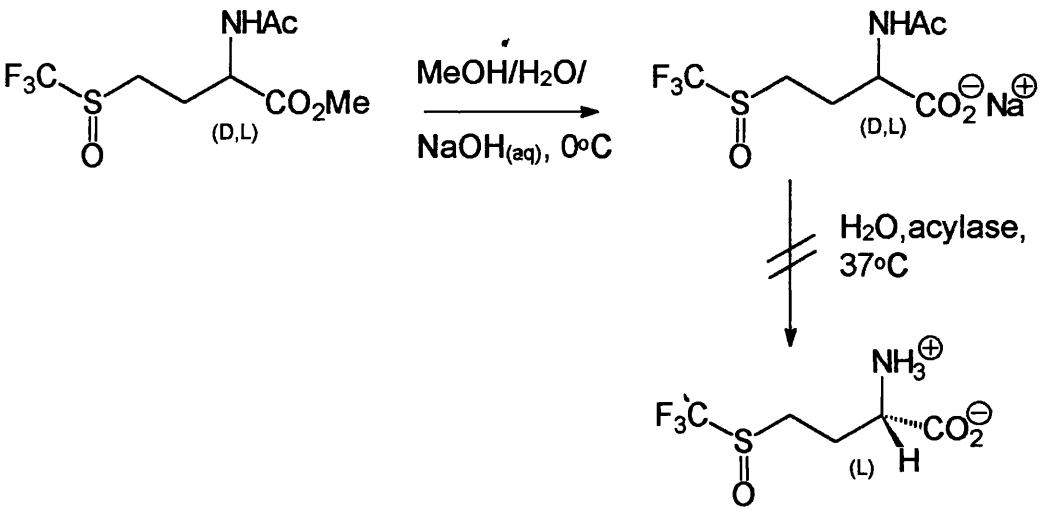
requires 232.0255 ($M^+ - COCH_3$)

Microanalysis (%)

found C 35.06, H 4.28, N 5.08

Requires C 34.90, H 4.36, N 5.09

L-Trifluoromethionine sulfoxide



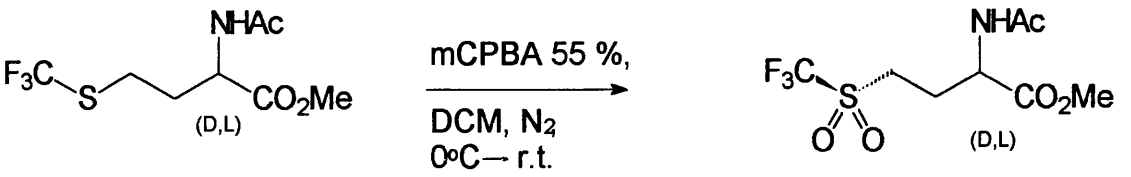
product has never been successfully prepared

adapted procedure: M.E. Houston and J.F. Honek, *J. Chem. Soc., Chem. Comm.,* 1989, 761

A single neck round bottomed flask (50ml) charged with N-acetyl-D,L-trifluoromethionine sulphoxide methyl ester (1.00g, 3.64mmol) dissolved in MeOH/H_2O (1:1, 30ml) was cooled to 0°C in an ice bath. To this was added an aqueous solution of NaOH (0.16g, 4.0mmol) and the mixture was stirred for 50min (the reaction was monitored by TLC to assure completion). The solvent was evaporated *in vacuo* giving the sodium salt. The sodium salt was dissolved in degassed distilled H_2O (150 ml) and the solution adjusted to pH 7.5 with 0.1M HCl. Porcine kidney acylase (1 mg, 1,430 units, supplied by Sigma Grade 1, product No. A3010) was added and the solution gently agitated overnight at 37°C in a water bath. The solution was adjusted to pH 5 with 6M HCl and activated charcoal (0.5g) added. The mixture was heated with stirring to 60°C for 10min and filtered. The filtrate was adjusted to pH 1.5 with 6M HCl and extracted with EtOAc (3 × 20ml). The aqueous layer, which did not test positive for the presence of free amino acid in ninhydrin spray spot tests on TLC film, was applied to a Dowex 50W RSO_3H column and eluted with H_2O

until the eluate was of the same pH as the distilled H_2O. The column was then eluted with 1.0M NH_4OH and the eluate collected and evaporated *in vacuo*. No product was isolated.

N-Acetyl-D,L-trifluoromethionine sulfone methyl ester



product has never before been prepared

procedure: M. Johnston, R. Raines, C. Walsh and R. A. Firestone, *J. Am. Chem. Soc.*, 1980, **102**, 4241

A flame dried round bottomed flask (25ml) equipped with side arm and septum cap, under N_2, was charged with trifluoromethionine methyl ester (1.00g, 4.93mmol) in distilled DCM (25ml) and stirred at 0°C in an ice bath. A solution of 55% mCPBA (3.12g, 10.0mmol) in distilled DCM (35ml), with added activated 4Å molecular sieves (2.0g) to reduce the overall water content, was added to the reaction flask via syringe over 1h. After 1h 30min at room temperature the solution was washed with $NaHCO_3$ solution (3 × 50ml), dried over Na_2SO_4, and filtered. The filtrate was evaporated in *vacuo* to give a yellow oil. The oil was applied to a silica column and eluted with EtOAc to give 0.98g (87.2%) of the title product as a clear oil.

^{1}H NMR ($CDCl_3$) δ

2.08 (3H, s, NHCOC<u>H</u>$_3$), 2.26 (1H, m, $CH_2\beta$), 2.50 (1H, m, $CH_2\beta$),
3.45 (2H, m, $CH_2\gamma$), 3.79 (3H, s, $CO_2C\underline{H}_3$), 4.77 (1H, m, CHα),
7.28 (1H, d, J = 7.3 Hz, N<u>H</u>Ac),

^{13}C NMR ($CDCl_3$) δ

22.6 (NHCO<u>C</u>H_3), 23.9 ($CH_2\beta$), 46.3 ($CH_2\gamma$), 50.4 (OCH_3), 52.8 (CHα),
119.2 (q, J_{CF} = 324.8 Hz, CF_3), 170.9 (NH<u>C</u>OCH_3), 171.4 (<u>C</u>O_2CH_3)

^{19}F NMR ($CDCl_3$) δ

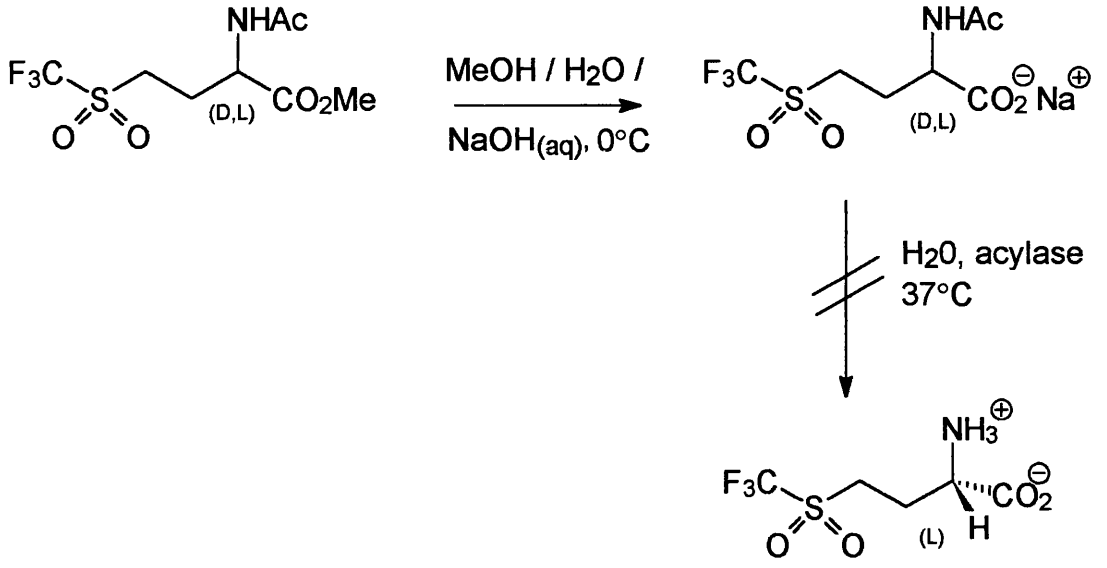
–78.3 (CF_3)

m/z

found 291.0403 (M^+)

requires 291.0388 (M^+)

L-Trifluoromethionine sulfone



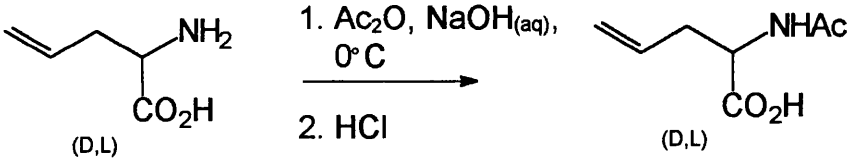
product has never successfully been prepared

adapted procedure: M.E. Houston and J.F. Honek, *J. Chem. Soc., Chem. Comm.*, 1989, 761

A single neck round bottomed flask (50ml) charged with N-acetyl-D,L-trifluoromethionine sulfone methyl ester (0.98g, 3.37mmol) was dissolved in MeOH/H_2O (1:1, 30ml) and cooled to 0°C in an ice bath. To this was added an aqueous solution of NaOH (0.15g, 3.74mmol) and the mixture was stirred for 50min (the reaction was monitored by TLC to assure completion). The solvent was evaporated *in vacuo* giving the sodium salt. (^{1}H NMR shows loss of the 3.76, 3H, s, $CO_2C\underline{H}_3$ signal). The sodium salt was dissolved in degassed distilled H_2O (190ml) and the solution adjusted to pH 7.5 with 0.1M HCl. Porcine Kidney acylase (1.3mg, 1,860 units, supplied by Sigma Grade 1, Product No. A3010) was added and the solution gently agitated overnight at 37°C in a water bath. The solution was adjusted to pH 5 with 6M HCl and activated charcoal (0.6g) added. The mixture was heated with stirring to 60°C for 10min and filtered. The filtrate was adjusted to pH 1.5 with 6M HCl and extracted with EtOAc (3 × 20ml). The aqueous layer did not test positive for the

presence of free amino acid in ninhydrin spray spot tests on TLC film. On evaporation of the aqueous layer *in vacuo* no product was isolated.

N-Acetyl-D,L-allylglycine



procedure: S. Black & N. G. Wright, *J. Biological Chemistry,* 1955, **39**, 213

D,L-Allylglycine (10.0g, 86.9mmol) and 2 M NaOH (45ml), in a conical flask, were stirred at 0°C in an ice bath. Distilled acetic anhydride (12.5ml, 132.5mmol) was added dropwise to the flask over the course of 1h. A saturated solution of NaOH was added during the same time period to keep the solution above
pH 9.5. 10M HCl (200ml) was then added and the solution extracted with EtOAc (3 × 150ml). The separated organic layer was dried over Na_2SO_4, filtered and evaporated in *vacuo*. N-acetyl-D,L-allylglycine crystallised as rectangular plates during the latter stages of distillation. The product was recrystallised from hot acetone by the addition of pet-ether 40-60 giving 10.13g (74.2%) of product. m.p. 115°C (literature value 114 - 117°C).

γmax (KBr disc) cm^{-1}

921, 992, 1674, 1738, 3427

^1H NMR ($CDCl_3$) δ

1.77 (3H, s, CH_3), 2.30 (2H, m, $CH_2\beta$),

4.17 (1H broad m, CHα),

4.91, 4.96, (2H, 2m, CH_2 vinyl), 5.53 (1H, m, CH vinyl)

^{13}C NMR ($CDCl_3$) δ

22.3 (CH_3), 35.7 ($CH_2\beta$), 53.3 (CHα), 119.6 (CH_2 vinyl), 133.4 (CH vinyl),

175.9 (CO_2H), 174.8 ($NH\underline{C}OCH_3$)

m/z

found 157.0728 (M^+), 112.0751 ($M^+ - CO_2$)

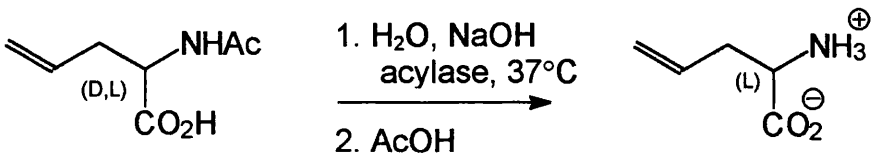
requires 157.1696 (M^+), 112.1518 ($M^+ - CO_2$)

microanalysis (%)

found C 53.41, H 7.03, N 8.87

requires C 53.50, H 7.01, N 8.92

L-Allylglycine



procedure: S. Black & N.G. Wright, *J. Biological Chemistry,* 1955, **39**, 213

A solution of N-acetyl-D,L-allylglycine (8.73g, 55.6mmol) dissolved in degassed distilled water (400ml) was adjusted to pH 7.5 with NaOH solution
Porcine kidney acylase (3.0mg, 4,290 units supplied by Sigma Grade I, Product No. A3010) was added, the conical flask was stoppered and the solution gently agitated overnight at 37°C in a water bath. The solution was adjusted to pH 5 with glacial acetic acid and activated charcoal (1.0g) added. The mixture was heated with stirring to 60°C for 10min before filtering. On concentration in *vacuo*, to about 50ml, the L-allylglycine began to crystallise out of solution. Crystallisation was completed by the addition of absolute EtOH (200ml). Recrystallisation from H_2O/EtOH (1:4) afforded L-allylglycine as white crystals in an overall yield of 2.52g, 39.4% (78.8% based on D,L mixture). m.p. 259°C (literature value 259°C).

γmax (KBr disc) cm^{-1}

920, 990, 1584, 1624, 2400 - 3220

^1H NMR (D_2O) δ

2.44 (2H, m, $CH_2\beta$), 3.62 (1H, t, J = 6 Hz, CHα), 5.09 (2H, m, CH_2 vinyl), 5.59 (1H, m, CH vinyl)

^{13}C NMR (D_2O) δ

35.6 ($CH_2\beta$), 54.7 (CHα),121.3 (CH_2 vinyl), 132.1 (CH vinyl), 174.9 (CO_2H)

$[\alpha]_D^{22}$ -35.0 ± 0.5 (H_2O)

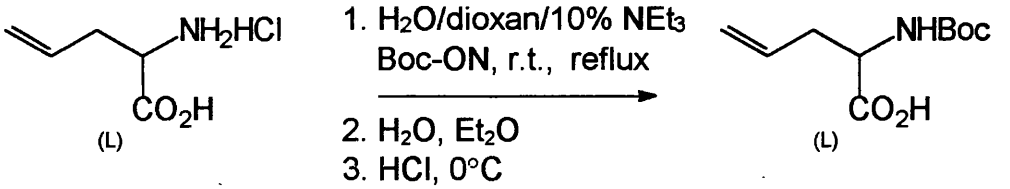
literature value $[\alpha]_D^{24}$ -37.1 ± 0.5 (H_2O)

m/z

found 115.0635 (M^+)

requires 115.1323 (M^+)

N-Boc-L-allylglycine



procedure: M. Johnston, R. Raines, C. Walsh & R. A. Firestone,
J. Am. Chem. Soc., 1980, **102**, 4241

To a round bottomed flask (25ml) charged with L-allylglycine (1.0g, 8.70mmol) and a mixed solution of H_2O / dioxan / 10% $NEt_{3(aq)}$ (1:1:1, 15ml) was added BOC-ON in a 10% excess (2.39g, 8.7mmol). The solution was then left to stir at r.t. for 4h before H_2O (20ml), then ether (25ml) were added. Following vigorous shaking the aqueous layer was separated and washed twice with ether (2 x 15ml). The aqueous layer was then acidified with stirring to pH 2 with 5 M HCl (5ml) at 0°C in an ice bath for 10min. The Boc protected amino acid was then extracted with EtOAc (3 × 25ml), dried over Na_2SO_4 and filtered. The filtrate was evaporated in *vacuo* and the resulting yellow oil crystallised from ether and washed with hexane giving 1.52g (81%) of the title product as a white crystals. m.p. 110°C (literature value 109 - 110°C).

γmax (KBr disc) cm^{-1}

912, 995, 1710, 1729, 2933, 3367

^1H NMR ($CDCl_3$, 200 MHz) δ

1.45 (9H, s, t-Bu), 2.55 (2H, m, $CH_2\beta$), 4.35 (1H, br, NH),

4.88 - 5.42 (3H, m, $CH\alpha$, CH_2 vinyl), 5.66 (1H, m, CH vinyl)

^{13}C NMR ($CDCl_3$, 200MHz) δ

28.2 ($C(\underline{C}H_3)_3$), 36.3 ($CH_2\beta$), 52.7 ($CH\alpha$), 80.3 ($\underline{C}(CH_3)_3$), 119.5 (CH_2 vinyl),

132.0 (CH vinyl), 155.5 ($\underline{C}O_2C(CH_3)_3$), 176.7 ($CO_2H$)

m/z

found 170.1178 ($M^+ - CO_2H$)

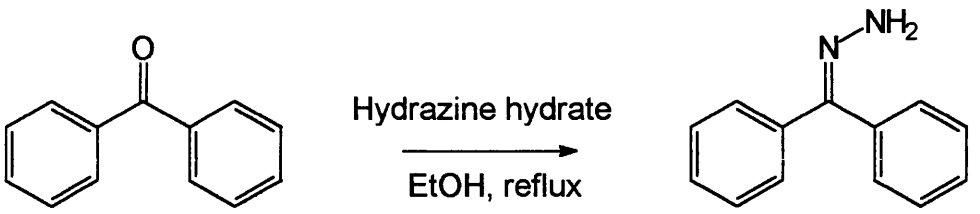
requires 170.2321 ($M^+ - CO_2H$)

microanalysis (%)

found C 55.62, H 7.84, N 6.50

requires C 55.81, H 7.91, N 6.51

Benzophenone hydrazone



procedure: Fieser & Fieser, *Reagents for Organic Chemistry Preparation,* 338

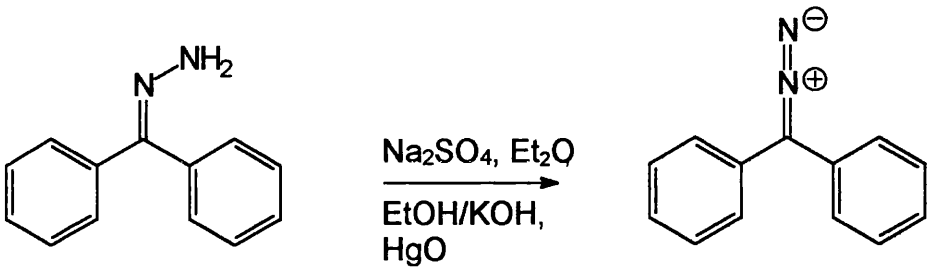
To a round bottomed flask (150ml) charged with distilled EtOH (23ml) was added benzophenone (6.12g, 33.6mol) and 100% hydrazine hydrate (6.31g, 12.6mol). The mixture was heated under reflux for 10h. On cooling in ice, colourless crystals of benzophenone hydrazone separated. 6.42g (97.4%) m.p. 97°C (literature value 98°C).

γmax (KBr disc) cm^{-1}
3202, 3422

^1H NMR ($CDCl_3$) δ
5.49 (s, 2H, NH_2), 7.59 - 7.25 (m, 10H, aromatic Hs)

microanalysis (%)
found C 79.34, H 6.18, N 14.45
requires C 79.59, H 6.18, N 14.29

Diphenyl diazomethane



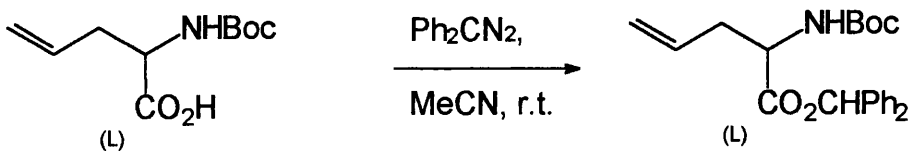
procedure: Fieser & Fieser, *Reagents for Organic Chemistry Preparation,* 338

Benzophenone hydrazone (350mg, 1.78mmol) and anhydrous Na_2SO_4 (0.4g, 2.82mmol) were added to a pressure bottle (100ml) charged with distilled Et_2O (5.4ml). Sequentially EtOH saturated with KOH (0.13ml) and yellow HgO (0.944g, 4.36mmol) were added. The bottle was sealed and wrapped in a wet towel before shaking for 1h, 15min. The solution was then filtered and evaporated in *vacuo.* The residue was taken up in pet ether 40-60 (15ml) and on evaporation this gave 339mg (98%) of the title product as crimson crystals. m.p. 31°C (literature value 30°C).

γmax (KBr disc) cm^{-1}
2020

^1H NMR ($CDCl_3$) δ
7.13 - 7.58 (m, 10H, aromatic Hs)

N-Boc-L-allylglycine benzhydryl ester



procedure: M. Johnston, R. Raines, C. Walsh & R. A. Firestone,
J. Am. Chem. Soc., 1980, **102**, 4241

N-Boc-L-allylglycine (3.23g, 15.0mmol) and diphenyldiazomethane (1.91g, 9.83 mmol) were dissolved in MeCN (175ml) in a conical flask. The solution was stirred at room temperature for 1h, during which time most of the crimson colour faded. The solvent was evaporated in *vacuo* and the product began crystallising out. The title product was recrystallised from ether/hexane (1:1) giving 3.82g (66.7%) of white crystals. m.p. 80 - 82°C (literature value 79 - 80°C).

γmax (KBr disc) cm^{-1}

1514, 1705, 1741

^1H NMR ($CDCl_3$) δ

1.49 (9H, s, $C(CH_3)_3$), 2.58 (2H, m, CHβ), 4.58 (1H, m, NH),

5.02 (3H, m, CHα, CH_2 vinyl), 5.60 (1H, m, CH vinyl), 6.82 (1H, s, $\underline{C}HPh_2$),

7.18 - 7.28 (10H, m, Ph_2)

^{13}C NMR ($CDCl_3$) δ

28.2 ($C(\underline{C}H_3)_3$), 36.6 ($CH_2\beta$), 52.9 (CHα), 77.9 ($\underline{C}HPh_2$), 79.8 ($\underline{C}(CH_3)_3$),

119.3 (CH_2 vinyl), 128.1 - 126.5 (Ph_2), 139.6 (C 131.9 (CH vinyl),

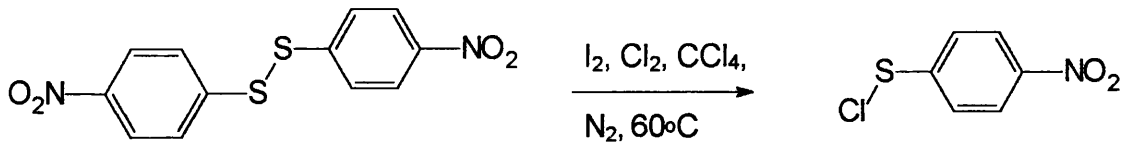
155.1 ($\underline{C}O_2C(CH_3)_3$), 171.1 ($\underline{C}O_2CHPh_2$)

m/z

found 381.1955 (M^+)

requires 381.4725 (M^+)

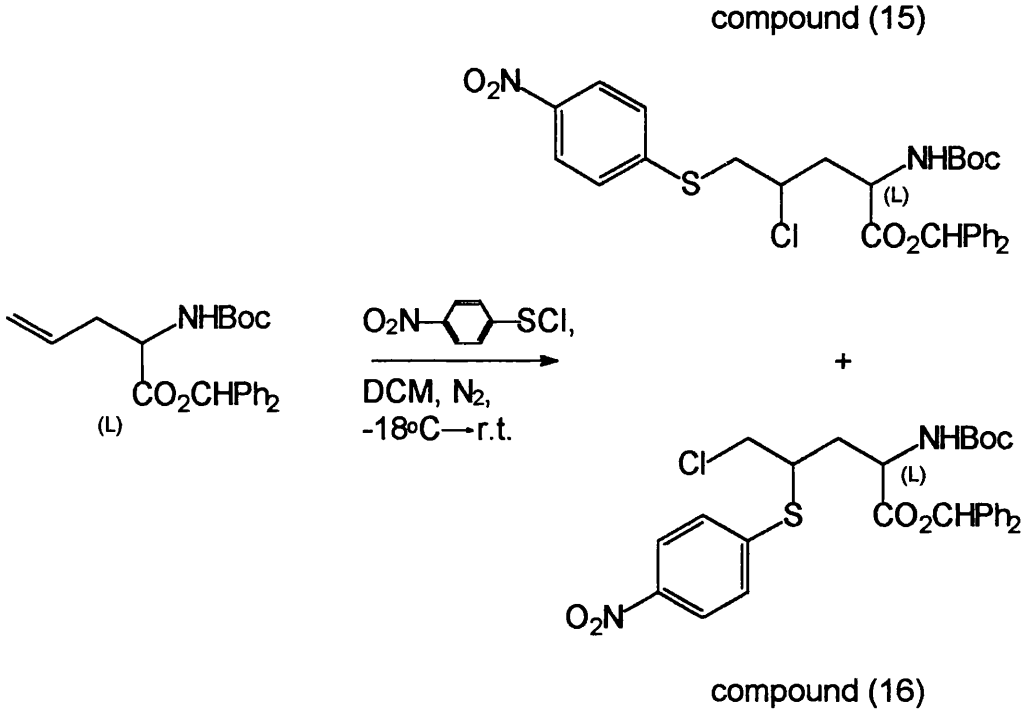
p-Nitrophenyl sulphenyl chloride



procedure: R. Katakai, *J. Chem. Soc., Perkin Trans 1.,* 2249 (1987)

A flame dried round bottomed flask (50ml) equipped with a three way tap, water condensor, N_2 balloon and side arm with septum, was charged with a suspension of p-nitrophenyl disulphide (0.540g, 1.75mmol), iodine (0.02g, 0.79mmol) and sieve dried CCl_4 (10ml). Liquefied Cl_2 (in excess) in sieve dried CCl_4 (15ml) was then added to the suspension via syringe and the mixture heated with stirring at 60°C in a water bath until the disulphide dissolved to give a yellow/orange clear solution (approximately 10min). The three way tap was then connected to a vacuum pump and the solvent evaporated. The newly prepared sulphenyl chloride crystallised out towards the end of evaporation and was stored under N_2 until required. (yield taken as 0.63g (95%) or above) m.p. 48°C (literature value 48°C).

2-N-Boc-4(5)-chloro-5(4)-(p-nitrophenyl sulfenyl) pentanoic acid benzhydryl ester



procedure: M. Johnston, R. Raines, C. Walsh & R. A. Firestone, *J. Am. Chem. Soc.*, 1980, **102**, 4241

A flame dried round bottomed flask (50ml) complete with side arm, septum cap and N_2 balloon was charged with N-Boc-L-allylglycine benzhdryl ester (4.27g, 11.21mmol) and distilled DCM (20ml). The mixture was cooled to –18°C with an ice / salt bath and p-nitrobenzene sulphenyl chloride (2.08g, 10.97mmol) in DCM (15ml) under N_2 was added dropwise over 30min via syringe. The solution was then allowed to warm to room temperature and stirred overnight. The solvent was evaporated in *vacuo* to give 4.82g of an oily residue. NMR showed a mixture of regioisomers (designated as compounds 15 & 16) in a 1:10 ratio (15:16). Separation could be achieved by repeated flash chromatography, eluting with EtOAc:$CHCl_3$, (50:1), but the partially purified mixture was usually carried forward since separation was easier at the sulphoxide stage. The overall yield for the mixture of regioisomers was 75.4%, no literature value was given.

γmax (KBr disc) cm^{-1}

854, 1514, 1597

NMR of 15

^1H NMR ($CDCl_3$) δ

1.28 (9H, s, t-Bu), 2.33 (2H, m, $CH_2\beta$), 3.50 (2H, m, CH_2S), 4.17 (1H, m, CHCl), 4.70 (1H, m, CHα), 5.27 (1H, m, NH), 6.93 (1H, s, C<u>H</u>Ph_2), 7.29 (10H, m, aromatic), 7.32 (d), 7.99 (4H, AA'BB', J = 8.9, 8.6, C_6H_4)

NMR of 16

^1H NMR ($CDCl_3$) δ

1.42 (9H, s, t-Bu), 1.92 (m), 2.59 (2H, m, $CH_2\beta$), 3.62 (3H, m, CH_2Cl & CHS), 4.70 (1H, m, CHα), 5.35 (1H, m, NH), 6.93 (1H, s, C<u>H</u>Ph_2), 7.29 (10H, m, aromatic), 7.32 (d), 7.99 (4H, AA'BB', J = 8.9, 8.6, C_6H_4)

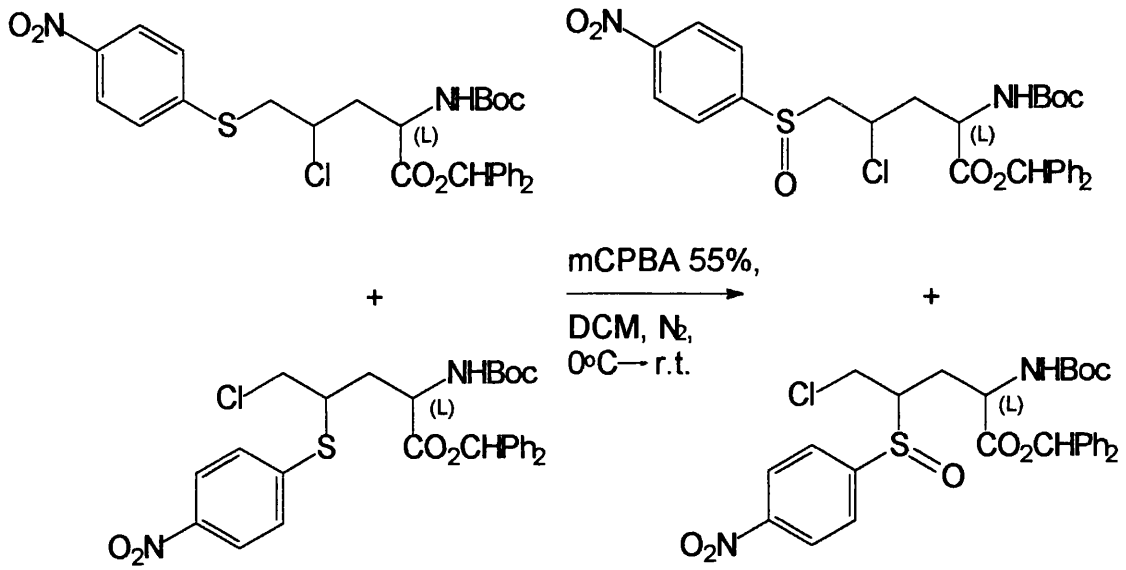
microanalysis of mixture of regioisomers 15 & 16 (%)

found C 60.92, H 5.29, N 4.81

requires C 61.00, H 5.26, N 4.91

2-N-Boc-4(5)-chloro-5(4)-(p-nitrophenyl sulfinyl) pentanoic acid benzhydryl ester

compound 17



compound 18

procedure: M. Johnston, R. Raines, C. Walsh & R. A. Firestone, *J. Am. Chem. Soc.*, 1980, **102**, 4241

A flame dried round bottomed flask (25ml) equipped with side arm and septum cap, under N_2, was charged with 15 & 16 (4.82g, 8.45mmol) in distilled DCM (100ml) and stirred at 0 °C in an ice bath. A solution of 55% mCPBA (2.65g, 8.45mmol) in distilled DCM (100ml), with activated 4Å molecular sieves to reduce the overall water content, was added to the reaction flask via syringe over 1h. After 30min at room temperature the solution was washed with $NaHCO_3$ solution (3 × 50ml), dried over Na_2SO_4, and filtered. The filtrate was evaporated in *vacuo* giving a yellow oil. The oil was applied to a silica column and eluted with EtOAc:$CHCl_3$ (50:1) to give products 17 & 18. Separation of 17 from 18 was achieved by repeated flash chromatography using the same eluants as before. Product 17 exists as two isomers I & II which were not separated. NMR data is given for 17 as a whole since I & II give almost the

same spectra. Only one isomer of 18 was isolated and analysed. The overall yield of 17 from 14 was 360mg (5.5%).

NMR of 17

^{1}H NMR ($CDCl_3$) δ

17I: 1.40 (9H, s, t-Bu), 2.12 (m), 2.33 (2H, m, $CH_2\beta$), 3.29 (2H, m, CH_2S), 4.60 (2H, m, CHCl, CHα), 5.25 (1H, d, J = 8.89 Hz, NH), 6.87 (1H, s, C<u>H</u>Ph_2), 7.32 (10H, s, Ph_2), 7.82 (d), 8.37 (4H, AA'BB', J = 8.74 Hz, C_6H_4)

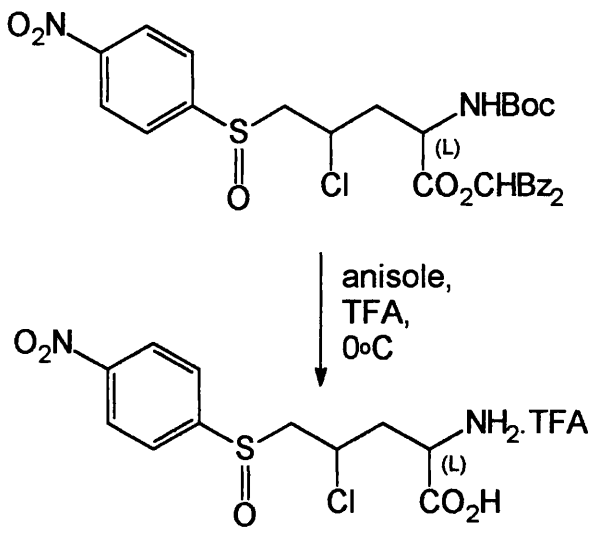
17II: 2.41 (2H, m, $CH_2\beta$), 3.19 (m), 3.38 (2H, m, CH_2S), 4.28 (1H, m, CHCl), 4.60 (1H, m, CHα), 5.39 (1H, d, J = 8.77 Hz, NH)

NMR of 18

^{1}H NMR ($CDCl_3$) δ

1.45 (9H, s, t-Bu), 2.49 (2H, m, $CH_2\beta$), 3.0 (1H, m, CHS), 3.6 (2H, m, CH_2Cl) 4.8 (1H, m, CHα), 5.49 (1H, m, NH), 6.91 (1H, s, C<u>H</u>Ph_2), 7.32 (10H, s, Ph_2), 7.89 (d), 8.30, (4H, AA'BB', J = 8.79 Hz, C_6H_4)

2-Amino-4-chloro-5-(p-nitrophenyl sulfinyl) pentanoic acid.TFA salt



procedure: M. Johnston, R. Raines, C. Walsh & R. A. Firestone,
J. Am. Chem. Soc., 1980, **102**, 4241

In a conical flask, 17I &17II (90mg, 0.153mmol) were dissolved in anisole (0.73ml, 6.7mmol) and treated with TFA (3.60ml, 46.7mmol) at 0°C in an ice bath with stirring for 12min. The reaction was monitored by TLC using ninhydrin spray to visualise free amino acid. Volatiles were then *removed in vacuo* at room temperature and a solution of H_2O (5ml) / DCM (5ml) was added. The mixture was transferred to a separating funnel and shaken vigorously; the aqueous layer was separated and evaporated in *vacuo* at room temperature, affording 49.9mg (102%) of the title amino acid protected as the TFA salt. The yield was not absolute and exceeded 100% due to the amino acid incorporating some water to give a glassy product.

^{1}H NMR (D_2O) δ

2.15 - 2.5 (m, 2H, $CH_2\beta$) 3.25 - 3.55 (m, 2H, CH_2S), 4.20 (m, 1H, CHCl), 4.41 (m, 1H, $CH\alpha$), 7.74 (m), 8.24 , (m, 4H, C_6H_4)

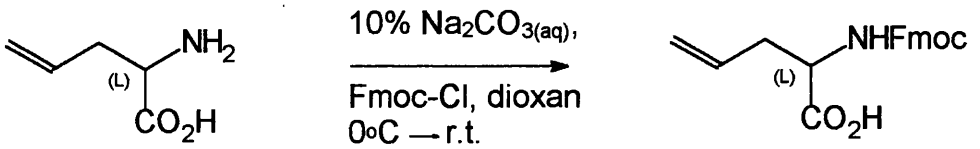
^{13}C NMR (D_2O) δ

38.2 ($CH_2\beta$), 52.0 (CHCl), 52.1 ($CH\alpha$), 63.0, 64.0 (CH_2S),

125.6, 126.3, 148.1, 148.6, (4 signals, CH aromatic)

150.7, 150.6 (2 quaternary aromatic), 163.6 (q, TFA), 171.8 (CO_2H)

N-Fmoc-L-allylglycine



product has never before been prepared

procedure: L.A. Carpino & G.Y. Han, *J. Org. Chem.*, **37**, 3404

In a conical flask, a mixture of L-allylglycine (1.38g, 12.0mmol) and a 10% Na_2CO_3 aqueous solution (45ml) were stirred at 0°C in an ice bath. A solution of Fmoc-Cl (3.12g, 12.06mmol) in dioxan (20ml) was added dropwise over 30min. The mixture was stirred for a further 5min at 0°C and then allowed to warm to room temperature and stirred for another 2h. The solution was then poured into H_2O (100ml) and extracted with Et_2O (2 × 50ml). The aqueous layer was separated and cooled in an ice bath before acidifying with 10M HCl to pH 1. The white precipitate was extracted with EtOAc (2 × 50ml), washed with H_2O (2 × 50ml), dried over Na_2SO_4 and filtered. The filtrate was evaporated in *vacuo* to give the title compound as a white residue. Recrystallisation from $MeNO_2$ gave 3.46g (85.6%) of the desired product as white crystals. m.p. 140°C (no literature value available).

γmax (KBr disc) cm^{-1}

1722, 2370 - 3200, 3402

^{1}H NMR ($CDCL_3$) δ

2.53 (2H, m, $CH_2\beta$), 4.20 (1H, t, J = 6.86 Hz,CHα), 4.46 (3H, m, $CHCH_2$ Fmoc), 5.14 (2H, m, CH_2 vinyl), 5.69 (1H, m, CH vinyl), 5.44 (1H, d, J = 8.12 Hz, NH), 7.24 -7.99 (8H, m, CH aromatic)

^{13}C NMR ($CDCL_3$) δ

36.3 ($CH_2\beta$), 47.0 ($CH_2\underline{C}H$, Fmoc), 53.1 (CHα), 67.2 (CH_2, Fmoc), 119.7 (CH_2 vinyl), 120.0 - 127.7 (4 signals, each 2 aromatic CH's), 131.64 (CH vinyl), 141.3, 143.6 (2CH's of the Fmoc group), 156.0 ($NHCO_2$),175.8 (CO_2H)

m/z

found 337.1342 (M^+)

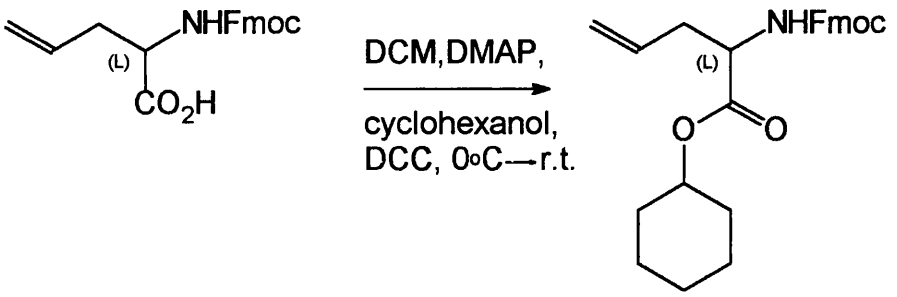
requires 337.3757 (M^+)

Microanalysis (%)

found C 70.75 , H 5.55, N 4.25

Requires C 71.22 , H 5.63, N 4.15

N-Fmoc-L-allylglycine cyclohexyl ester



product has never before been prepared

adapted procedure: J.P. Tam, T. Wong, M.W. Riemen, F. Tjoeng & R.B. Merrifield, *Tetrahedron. Lett.,* 1979, 4033

A single neck round bottomed flask (100ml) charged with N-Fmoc-L-allylglycine (1.99g, 5.92mmol), anhydrous DCM (50ml), DMAP (46mg, 0.38mmol) and cyclohexanol (1.40g,14.0mmol) was stirred at 0°C in an ice bath. DCC (1.22g, 5.92mmol) was added to the reaction flask and the mixture stirred for a further 5min at 0°C and 3h at room temperature. The mixture was filtered and the filtrate evaporated in *vacuo.* The residue was taken up in DCM (30ml) and refiltered as necessary, washed with 0.5M HCl (2 × 25ml) and an aqueous solution of $NaHCO_3$ (2 × 25ml), dried over Na_2SO_4 and filtered. The filtrate was evaporated in *vacuo* and the resulting yellow oil applied to a silica column, eluting with DCM. This gave a clear oil, which recrystallised from DCM giving 1.66g (67%) of pure product. m.p.105 - 106 °C.

γmax (KBr disc) cm^{-1}

1686, 1714, 1738, 2856, 2936, 3324

^1H NMR ($CDCl_3$) δ

1.27 - 1.79 (m, 10H, 5 x CH_2 cyclohexyl), 2.55 (m, 2H, $CH_2\beta$),

4.23 (t, 1H, J = 6.93 Hz ,$CH\alpha$), 4.43 (m, 3H, $CHCH_2$ Fmoc),

4.81 (m, 1H, $CO_2CHC_5H_{10}$), 5.11 (d, 2H, J = 11.72 Hz, CH_2 vinyl),

5.54 (d, 1H, J = 8.14 Hz, NH), 5.68 (m, 1H, CH vinyl),

7.21 - 7.73 (m, 8H aromatic)

^{13}C NMR ($CDCl_3$) δ

23.5, 25.2, 31.4 (5 x CH_2, C_6H_{10}), 36.3 ($CH_2\beta$), 47.1 (CH_2CH, Fmoc),

53.4 (CH, vinyl), 67.0 ($CHCH_2$, Fmoc), 74.0 (CH, cyclohexyl),

119.2 (CH_2, vinyl), 120.0, 125.1, 127.0, 127.7 (4 signals, each 2 aromatic CH's)

132.2 (CH, vinyl), 141.3, 143.8 (2CH's of the Fmoc group), 155.7 ($NHCO_2$),

171.1 (CO_2H)

m/z

found 419.2117 (M^+)

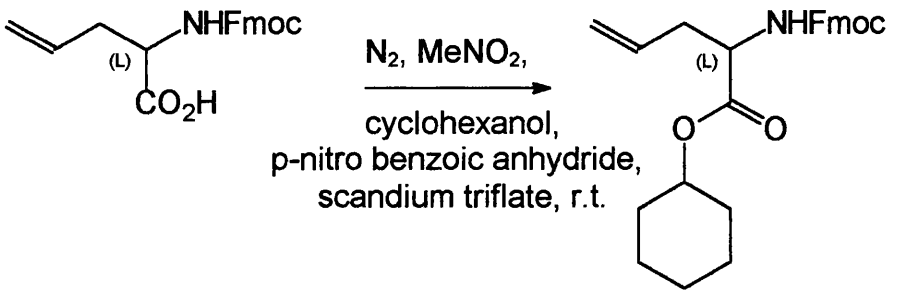
requires 419.5214 (M^+)

Microanalysis (%)

found C 74.66, H 7.19, N 3.15

Requires C 74.50, H 7.40, N 3.34

N-Fmoc-L-allylglycine cyclohexyl ester

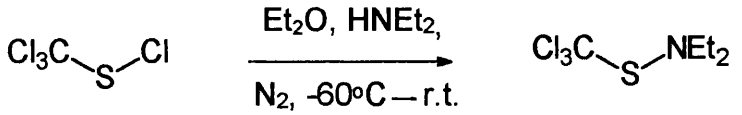


procedure: K. Ishihara, M. Kubota, H. Kurihara & H. Yamamoto,
J. Am. Chem. Soc., 1995, **117**, 4413

A flame dried 50ml round bottomed flask equipped with side arm and septum cap, under N_2, was charged with N-Fmoc-L-allylglycine (0.625g, 1.86mmol) in distilled $MeNO_2$ (20ml). Sequentially cyclohexanol (0.169g, 1.69mmol) in $MeNO_2$ (10ml), *p*-nitrobenzoic anhydride (0.8g, 2.5mmol) in $MeNO_2$ (15ml) and scandium triflate (1mg, 0.003mmol) in $MeNO_2$ (1ml) were added to the reaction flask via syringe. The reaction mixture was stirred overnight and in the morning a white precipitate could be seen. The reaction was quenched with $NaHCO_3$ solution (40ml), the product extracted with Et_2O (50ml), dried over Na_2SO_4 and filtered. The filtrate was evaporated in *vacuo* and the crude product column chromatographed on silica, eluting with DCM to give 1.62g (62%) of the pure product.

Analytical data as before.

Trichloromethyl-(N-diethyl)-sulfenamide



procedure: H. Lecher & F. Holscheneider, *Chem. Ber.*,1924, **57**, 755

A flame dried round bottomed flask (50ml) equipped with side arm and septum cap, under N_2, was charged with trichloromethyl sulfenylchloride (8.0g, 43mmol) in distilled Et_2O (100ml). The mixture was stirred at –60°C and diethylamine (6.64g, 90.8mmol) was added over the course of 30min. The mixture warmed to room temperature and stirring continued for a further 3h. The white precipitate was removed by filtering and the filtrate evaporated in *vacuo* giving 9.09g (95%) of the title product as a yellow liquid.

^{1}H NMR ($CDCl_3$) δ

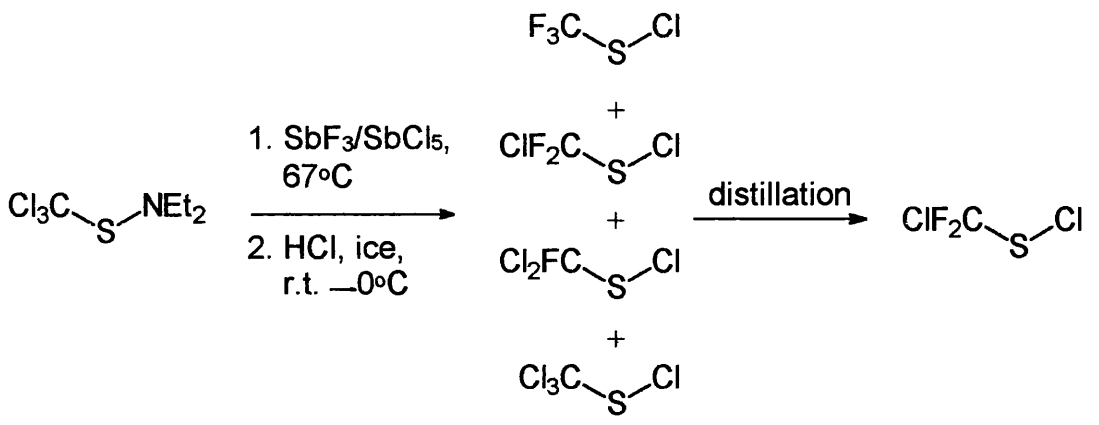
3.55, 3.34 (m, 4H, 2 × CH_2), 1.24 (t, 3H, J = 7.15 Hz, CH_3)

^{13}C NMR ($CDCl_3$,) δ

51.8 (CH_2), 14.4 (CH_3)

m/z	Cl_3CSNEt_2
found	104.0534 (M^+ –CCl_3)
requires	104.1904 (M^+ –CCl_3)

Difluorochloromethane sulfenyl chloride



procedure: N.N. Yarovenko, S.P. Motornyl, A. S. Vasil'eva & T. P. Gershzon, *J. Gen. Chem. U.S.S.R.*, 1959, **29**, 2129

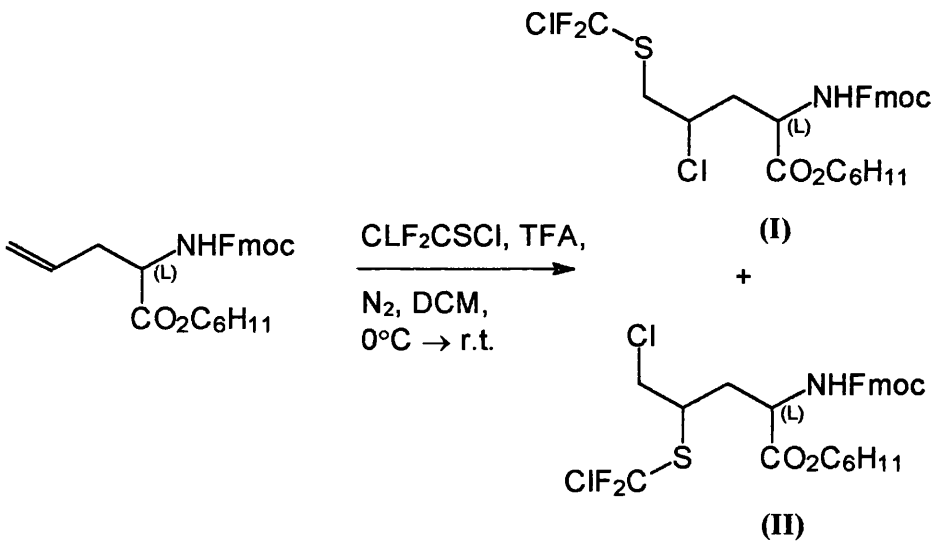
A 3 neck round bottomed flask (50ml) fitted with a stirrer and a thermometer was charged with trichloromethyl-(N-diethyl)-sulfenamide (6.9g, 31mmol), SbF_3 (4.14g, 18.1mmol) and $SbCl_5$ (0.14g, 0.47mmol). The mixture was stirred at 67°C and kept at this temperature until the lower layer became quite black (1h 45min). The upper layer was separated from the tars, transferred to a 25ml conical flask and saturated with dry $HCl_{(g)}$ (in excess) for 2h 30min. The reaction mixture was then vigorously stirred and cooled in an ice bath. Finely crushed ice was added and the yellow oily layer was quickly separated and dried over $CaCl_2$. The different sulfenyl chlorides were separated by Kugelrohr distillation. F_3CSCl b.p. 0°C (could not be isolated), ClF_2CSCl b.p. 49°C (1.02g (22%)), Cl_2FCSCl b.p. 99°C (0.38 g (11%)), Cl_3CSCl b.p. 149°C (not isolated).

ClF_2CSCl

^{19}F NMR ($CDCl_3$) δ

–37.1 (s, 2F)

2-N-Fmoc-4(5)-chloro-5(4)-(chlorodifluoromethylsulfenyl)pentanoic acid cyclohexyl ester



product has never before been prepared

procedure: A. Haas, M. Lieb & Y. Zhang, *J. Fluorine Chem.,* 1985, 203

A flame dried 50ml round bottomed flask with side arm, septum cap and N_2 balloon, was charged with N-Fmoc-L-allylglycine cyclohexyl ester (0.88g, 2.10mmol) in distilled DCM (15ml) and 100% TFA (0.04g, 0.35mmol). The solution was stirred at 0°C in an ice bath and chlorodifluoromethyl sulfenyl chloride (0.31g, mmol) in distilled DCM (10ml) was added over the course of 30min. The mixture warmed to room temperature and stirred overnight. The solvent and TFA were evaporated in *vacuo* leaving a golden oil. Starting material and the two regioisomers were isolated by preparative thin layer chromatography using DCM / hexane (10:1) as the eluant to isolate 230mg (19.1%) of regioisomer I.. Regioisomer I could not be fully isolated from the starting material and regioisomer II at this stage. Integration of the ^{1}H NMR spectrum of the unpurified product mixture indicated that the ratio of regioisomers I : II was approximately 2:3.

Compound I:

^{1}H NMR ($CDCl_3$) δ

1.25 - 1.89 (m, 10H, 5 × CH_2 cyclohexyl), 1.90-2.59 (m, 2H, $CH_2\beta$),

3.74 (m, Hz, 2H, CH_2SCF_2Cl), 4.02 (m, 1H, CHCl),

4.22 (t, 1H, J = 6.86 Hz ,CHα), 4.30 - 4.74 (m, 3H, $CHCH_2$ Fmoc),

4.86 (m, 1H, $CO_2CHC_5H_{10}$), 5.52 (d, 1H, J = 8.14 Hz, NH),

7.26 - 7.79 (m, 8H aromatic)

^{13}C NMR ($CDCl_3$) δ

23.6, 25.2, 31.4 (5 x CH_2 cyclohexyl), 36.3 ($CH_2\beta$), 44.7 (CHCl),

47.3 (CH_2S), 51.7, CH cyclohexyl), 67.0, ($CH\underline{C}H_2$, Fmoc),

74.0 ($\underline{C}HCH_2$, Fmoc), 120.0, 125.1, 127.0, 127.7, (4 signals, 8 × CH aromatic),

132.2 (t, J = 337.2Hz, CF_2Cl), 141.3, 143.8 (2 aromatic CH's),

155.7 ($NHCO_2$), 171.1 ($\underline{C}O_2CH$)

^{19}F NMR ($CDCl_3$) δ

-25.81 (s, 2F,-CH_2SCF_2Cl)

Compound II:

^{1}H NMR ($CDCl_3$) δ

1.22 - 1.81 (m, 10H, 5 × CH_2 cyclohexyl), 2.28 (m, 2H, $CH_2\beta$),

3.51 - 3.77 (m, Hz, 2H, CH_2Cl), 3.93 (m, 1H, $CHSCF_2Cl$),

4.21 (t, 1H, J = 6.80 Hz ,CHα), 4.34 - 4.70 (m, 3H, $CHCH_2$ Fmoc),

4.83 (m, 1H, $CO_2CHC_5H_{10}$), 5.41 (d, 1H, J = 8.86 Hz, NH),

7.24 - 7.77 (m, 8H aromatic)

^{13}C NMR ($CDCl_3$) δ

23.5, 25.2, 31.3 (5 x CH_2 cyclohexyl), 34.2 ($CH_2\beta$), 45.0 (CHS), 47.10 (CH∝),

47.3 (CH_2Cl), 51.8, CH cyclohexyl), 67.1, ($CHCH_2$, Fmoc),

74.6 (CH, Fmoc), 120.0, 125.1, 127.1, 127.8, (4 signals, 8 × CH aromatic),

128.0 and 134.1 (2d, J = 315Hz, CF_2Cl), 141.3, 143.8 (2 aromatic CH's),

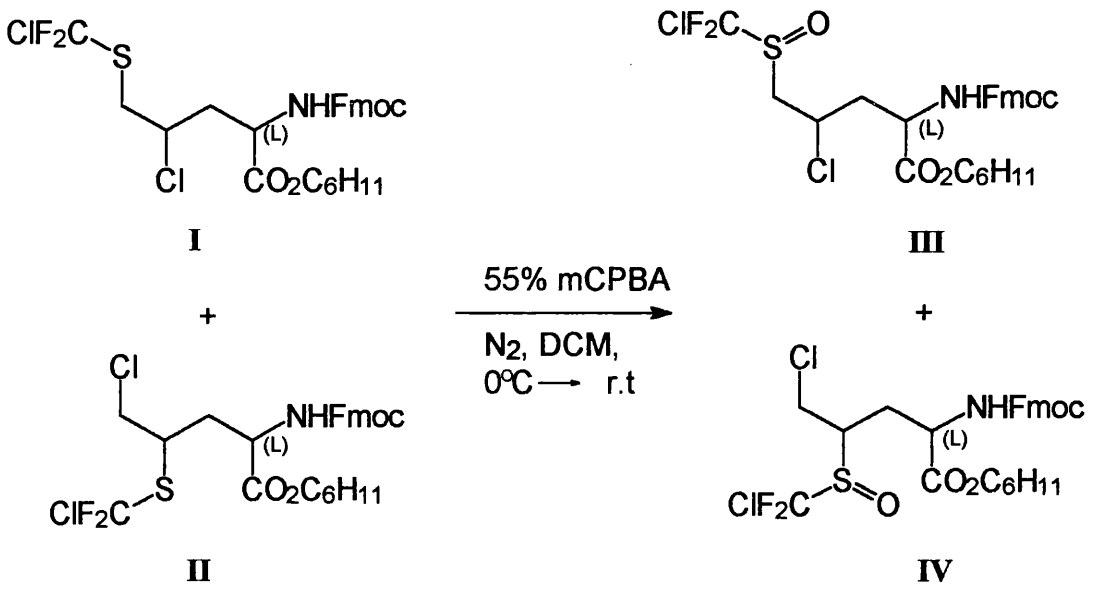
156.0 ($NHCO_2$), 170.7 ($\underline{C}O_2CH$)

^{19}F NMR ($CDCl_3$) δ

-25.30 and -26.88 (2m, 2F, $CClF_2S$), within the 2 multiplets we can distinguish 2 doublets: -25.20 (d, J = 136.1 ,z -CCl(F)FS),

-27.05 (d, J = 136.1Hz, -CClF(F)S)

2-N-Fmoc,4(5),chloro-5(4)(chlorodifluoromethylsulfinyl)pentanoic acid cyclohexyl ester



product has never before been prepared

procedure: M. Johnston, R. Raines, C. Walsh & R. A. Firestone, *J. Am. Chem. Soc.,* 1980, **102**, 4241

A flame dried round bottomed flask (25ml) equipped with side arm and septum cap, under N_2, was charged with I & II (0.36g, 0.63mmol) in distilled DCM (10ml) and stirred at 0°C in an ice bath. A solution of 55% mCPBA (0.22g, 0.63mmol) in distilled DCM (10ml), with added drying sieves to reduce the overall water content, was added to the reaction flask via syringe over 1h. After 30min at room temperature the solution was washed with $NaHCO_3$ solution (2 × 10ml), dried over Na_2SO_4, and filtered. The filtrate was evaporated in *vacuo* giving a yellow oil. The oil was applied to a silica column and eluted with EtOAc:$CHCl_3$ (50:1) to give 121mg of product III (21.2% yield, or 53% based on the 2:3 ratio of I : II). Product III exists as a number of diastereoisomers which were not separated.

Compound III:

^{13}C NMR ($CDCL_3$) δ

23.5, 25.1, 31.3 (5 x CH_2 cyclohexyl), 39 - 40.6 (m, $CH_2\beta$), 41.9 (CHCl)

47.0 ($CH\alpha$), 50.6 - 51.0 (CH_2SO), 51.5, (CH cyclohexyl), 67.2, ($CH\underline{C}H_2$, Fmoc),

75.1 ($\underline{C}HCH_2$, Fmoc), 120.0, 124.8, 127.1, 127.7, (4 signals, 8 × CH aromatic)

129.7 & 130.1(2t, J_1 = 2.0Hz & J_2 = 2.1Hz, C(F)FCl & CF(F)Cl),

141.3, 143.4 (2 aromatic CH's), 155.6 ($NHCO_2$), 170.5 ($\underline{C}O_2CH$)

^{19}F NMR ($CDCl_3$) δ

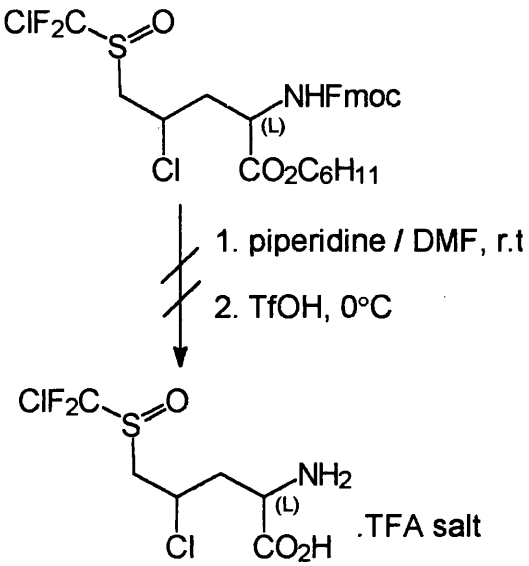
$-CF_2Cl$

-54.6, (d of d, J_1 = 558.5 Hz & J_2 = 149.2Hz),

-60.2, (t, J = 455.9Hz),

-58.6 to -60.2 (m)

2-amino-4-chloro-5-(chlorodifluoromethylsulfinyl)pentanoic acid TFA salt



product has never successfully been prepared

Procedures: L.A. Carpino & G.Y. Han, *J. Org. Chem.*, **37**, 3404 and J.P. Tam, Tai-Wai Wong, M.W. Rieman, Foe-S. Tjoeng and R.B. Merrifield, *Tett. Letts.*, 4033 (1979)

In a conical flask, III(A) and III(B) (100mg, 0.17mmol) were dissolved in DMF (5ml) and treated with a 20% piperidine / DMF solution (0.17mmol of piperidine) and stirred at r.t. for 1min, the reaction vessel was cooled to 0°C and methanesulfonic acid (19.2mg, 0.20mmol) along with 2 drops of TFA was then added to the reaction flask. The reaction was stirred at 0°C for 1h and monitored by TLC using ninhydrin spray to visualise free amino acid. No free amino acid was observed. Distilled water (5ml) was added to the reaction mixture and the mixture transferred to a separating funnel. After shaking vigorously, the layers were separated and evaporated in *vacuo* at room temperature. Starting material was recovered (confirmed by NMR). The deprotection was then attempted a 2nd time. Starting material III amounting to 55mg (55%) was the only product recovered.

BIOLOGICAL TESTING

General Methods:

Drug tests were performed in duplicate using minimum essental medium (designated HOMEM medium) on microtitre plates. The parasite starting density in each test were 10^4 cells ml^{-1}. The test cultures comprised of 20μl drug + 180μl culture. Apart from the control leg where 20μl H_2O replaced the 20μl drug. Final cell counts were estimated using an inverted microscope.

To observe the range of activity of individual drugs a variety of parasitic protozoa were tested. The Trichomonads, Giardia and Entamoeba are anaerobic protozoa all the others tested were aerobic protozoa.

The Trichomonads were tested both anaerobically and aerobically as it is known that cell lines resistant to the standard drug (metronidazole) show resistance only when oxygen is present. However for most drugs it appears to make no difference.

Key to parasitic growth densities:

+3	=	high
+2	=	medium
+1	=	low
+	=	very low
-	=	none

L-Trifluoromethionine and L-Trifluoromethyl cysteine

Biological activity profiles versus *Trichomonas vaginalis*

Growth inhibition results for *T. vaginalis* in the presence of varying concentrations of L-Trifluoromethionine, L-Trifluoromethyl cysteine and the known growth inhibitor Metronidazole.

Drug	Metronidazole		L-Trifluoromethionine		L-Trifluoromethyl cysteine	
Conc^{n} μg ml^{-1}	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
100	-	-	-	-	-	-
50	-	-	-	-	-	-
25	-	-	-	-	-	-
10	-	-	+	+	-	-
5	-	-	+	+	+	+
2.5	-	-	+1	+1	+1	+1
1	+	+	+2	+2	+1	+1
control	+3	+3	+3	+3	+3	+3

Test conditions: 20μl aqueous drug solution + 180μl culture, 24h, aerobic incubation, 37°C, microtitre plate. Ref (84) 9/9/93

Biological activity profile versus *Trichomonas foetus*

Growth inhibition results for *T. foetus* in the presence of varying concentrations of L-Trifluoromethionine, L-Trifluoromethyl cysteine and the known growth inhibitor Metronidazole.

Drug	Metronidazole		L-Trifluoromethionine		L-Trifluoromethyl cysteine	
Conc^{n} μg ml^{-1}	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
100	-	-	+2	+2	+	+
50	-	-	+2	+2	+	+
25	-	-	+3	+3	+1	+1
10	-	-	+3	+3	+1	+1
5	-	-	+3	+3	+2	+2
2.5	-	-	+3	+3	+3	+3
1	+	+	+3	+3	+3	+3
control	+3	+3	+3	+3	+3	+3

Test conditions: 20μl aqueous drug solution + 180μl culture, 24h, aerobic incubation, 37°C, microtitre plate. Ref (84) 15/9/93

Biological activity profile versus *Entamoeba invadens*

Growth inhibition results for *E. invadens* in the presence of varying concentrations of L-Trifluoromethionine and L-Trifluoromethyl cysteine.

Drug	L-Trifluoromethionine		L-Trifluoromethyl cysteine	
Conc^{n} μg ml^{-1}	Rep 1	Rep 2	Rep 1	Rep 2
100	+1	+1	+1	+1
50	+2	+2	+2	+2
25	+3	+3	+2	+2
10	+3	+3	+2	+2
5	+3	+3	+3	+3
2.5	+3	+3	+3	+3
1	+3	+3	+3	+3
control	+3	+3	+3	+3

Test conditions: 20μl aqueous drug solution + 180μl culture, 48h, anaerobic incubation, 25°C, microtitre plate. Ref (84) 9/12/93

Biological activity profile versus *Entamoeba invadens*

Growth inhibition results for *E. invadens* in the presence of varying concentrations of L-Trifluoromethionine and L-Trifluoromethyl cysteine.

Drug	L-Trifluoromethionine		L-Trifluoromethyl cysteine	
Conc^{n} μg ml^{-1}	Rep 1	Rep 2	Rep 1	Rep 2
100	+1	+1	-	-
50	+2	+2	+	+
25	+2	+2	+1	+1
10	+3	+3	+2	+2
5	+3	+3	+3	+3
2.5	+3	+3	+3	+3
1	+3	+3	+3	+3
control	+3	+3	+3	+3

Test conditions: 20μl aqueous drug solution + 180μl culture, 144h, anaerobic incubation, 25°C, microtitre plate. Ref (84) 13/12/93

Biological activity profile versus macrophages

Growth inhibition results for macrophages in the presence of varying concentrations of L-Trifluoromethionine and L-Trifluoromethyl cysteine.

Drug	L-Trifluoromethionine		L-Trifluoromethyl cysteine	
Conc^{n} μg ml^{-1}	Rep 1	Rep 2	Rep 1	Rep 2
100	+3	+3	+1	+1
50	+3	+3	+1	+1
25	+3	+3	+2	+2
10	+3	+3	+3	+2
5	+3	+3	+3	+3
2.5	+3	+3	+3	+3
1	+3	+3	+3	+3
control	+3	+3	+3	+3

Test conditions: 20μl aqueous drug solution + 180μl culture, 48h, 5% CO_2 incubation, 32°C, microtitre plate. Ref (84) 9/12/93

Biological activity profile versus macrophages

Growth inhibition results for macrophages in the presence of varying concentrations of L-Trifluoromethionine and L-Trifluoromethyl cysteine.

Drug	L-Trifluoromethionine		L-Trifluoromethyl cysteine	
Conc^{n} μg ml^{-1}	Rep 1	Rep 2	Rep 1	Rep 2
100	+3	+3	-	-
50	+3	+3	+	+
25	+3	+3	+1	+1
10	+3	+3	+2	+2
5	+3	+3	+3	+3
2.5	+3	+3	+3	+3
1	+3	+3	+3	+3
control	+3	+3	+3	+3

Test conditions: 20μl aqueous drug solution + 180μl culture, 144h, 5% CO_2 incubation, 32°C, microtitre plate. Ref (84) 13/12/93

Biological activity profiles versus *Leishmania mexicana*

Growth inhibition results for *L. mexicana* in the presence of varying concentrations of L-Trifluoromethionine, L-Trifluoromethyl cysteine and Metronidazole.

Drug	Metronidazole		L-Trifluoromethionine		L-Trifluoromethyl cysteine	
Conc^{n} μg ml^{-1}	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
100	+3	+3	-	+	-	-
50	+3	+3	+	+	-	-
25	+3	+3	+1	+	-	-
10	+3	+3	+1	+1	-	-
5	+3	+3	+2	+2	-	-
2.5	+3	+3	+2	+2	+	+
1	+3	+3	+2	+2	+	+
control	+3	+3	+3	+3	+3	+3

Test conditions: 20μl aqueous drug solution + 180μl culture, 72h, aerobic incubation, 25°C, microtitre plate. Ref (84) 11/10/93

Biological activity profile versus *Leishmania major*

Growth inhibition results for *L. major* in the presence of varying concentrations of L-Trifluoromethionine, L-Trifluoromethyl cysteine and Metronidazole.

Drug	Metronidazole		L-Trifluoromethionine		L-Trifluoromethyl cysteine	
Conc^{n} μg ml^{-1}	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
100	+3	+3	+1	+1	-	-
50	+3	+3	+2	+2	-	-
25	+3	+3	+3	+3	-	-
10	+3	+3	+3	+3	+	+
5	+3	+3	+3	+3	+	+
2.5	+3	+3	+3	+3	+1	+
1	+3	+3	+3	+3	+1	+1
control	+3	+3	+3	+3	+3	+3

Test conditions: 20μl aqueous drug solution + 180μl culture, 72h, aerobic incubation, 25°C, microtitre plate. Ref (84) 11/10/93

Biological activity profiles versus *Leishmania donovani*

Growth inhibition results for *L. donovani* in the presence of varying concentrations of L-Trifluoromethionine, L-Trifluoromethyl cysteine and Metronidazole.

Drug	Metronidazole		L-Trifluoromethionine		L-Trifluoromethyl cysteine	
Conc^{n} μg ml^{-1}	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
100	+3	+3	+	+	-	-
50	+3	+3	+	+	-	-
25	+3	+3	+	+	-	-
10	+3	+3	+1	+1	-	-
5	+3	+3	+3	+2	-	-
2.5	+3	+3	+3	+3	+	+
1	+3	+3	+3	+3	+	+
control	+3	+3	+3	+3	+3	+3

Test conditions: 20μl aqueous drug solution + 180μl culture, 72h, aerobic incubation, 25°C, microtitre plate. Ref (84) 11/10/93

Biological activity profile versus *Leishmania tarentolae*

Growth inhibition results for *L. tarentolae* in the presence of varying concentrations of L-Trifluoromethionine and L-Trifluoromethyl cysteine.

Drug	L-Trifluoromethionine		L-Trifluoromethyl cysteine	
Conc^{n} μg ml^{-1}	Rep 1	Rep 2	Rep 1	Rep 2
100	+	+	-	-
50	+1	+1	-	-
25	+2	+2	-	-
10	+3	+3	-	-
5	+3	+3	-	-
2.5	+3	+3	-	-
1	+3	+3	+	+
control	+3	+3	+3	+3

Test conditions: 20μl aqueous drug solution + 180μl culture, 48h, aerobic incubation, 25°C, microtitre plate. Ref (84) 2/12/93

Biological activity profile versus *Giardia lamblia*

Growth inhibition results for *G. lamblia* in the presence of varying concentrations of L-Trifluoromethionine and L-Trifluoromethyl cysteine.

Drug	L-Trifluoromethionine		L-Trifluoromethyl cysteine	
Conc^{n} $\mu g\ ml^{-1}$	Rep 1	Rep 2	Rep 1	Rep 2
100	+3	+3	-	-
50	+3	+3	+1	+1
25	+3	+3	+1	+1
10	+3	+3	+2	+2
5	+3	+3	+2	+2
2.5	+3	+3	+3	+3
1	+3	+3	+3	+3
control	+3	+3	+3	+3

Test conditions: 20µl aqueous drug solution + 180µl culture, 48h, anaerobic incubation, 37°C, microtitre plate. Ref (84) 2/12/93

L-Trifluoromethionine

Biological activity profiles versus a variety of micro-organisms

Growth inhibition results for a variety of micro-organisms in the presence of varying concentrations of L-Trifluoromethionine.

Drug	T. augusta		C. fasciculata		H.m. muscanum		H.m. ingenoplastis		L. tarentolae		T. pyriforris	
Conc^{n} μg ml^{-1}	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
100	+1	+1	+3	+3	+2	+2	+3	+3	↑		+3	+3
50	+1	+2	+3	+3	+2	+2	+3	+3	↑		+3	+3
25	+1	+1	+3	+3	+3	+3	+3	+3	DEAD		+3	+3
10	+1	+1	+3	+3	+3	+3	+3	+3	Did		+3	+3
5	+1	+1	+3	+3	+3	+3	+3	+3	Not		+3	+3
2.5	+1	+1	+3	+3	+3	+3	+3	+3	Grow		+3	+3
1	+3	+1	+3	+3	+3	+3	+3	+3	↓		+3	+3
control	+3	+3	+3	+3	+3	+3	+3	+3	↓		+3	+3

Test conditions: 20μl aqueous drug solution + 180μl culture, 48h, aerobic incubation, 25°C, microtitre plate. Ref (84) 29/10/93

Drug	T. augusta		C. fasciculata		H.m. muscanum		H.m. ingenoplastis		L. tarentolae		T. pyriforris	
Conc^{n} μg ml^{-1}	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
100	-	-	+	+	+2	+2	+3	+3	↑		+3	+3
50	-	+3	+	+	+2	+2	+3	+3	↑		+3	+3
25	-	-	+	+	+2	+3	+3	+3	DEAD		+3	+3
10	-	-	+	+	+3	+3	+3	+3	Did		+3	+3
5	-	-	+	+	+3	+3	+3	+3	Not		+3	+3
2.5	-	-	+	+	+3	+3	+3	+3	Grow		+3	+3
1	+3	-	+	+	+3	+3	+3	+3	↓		+3	+3
control	+3	+3	+	+	+3	+3	+3	+3	↓		+3	+3

Test conditions: 20μl aqueous drug solution + 180μl culture, 120h, aerobic incubation, 25°C, microtitre plate. Ref (84) 1/11/93

Key: T = trichomonas, C = Crithdia, Hm = Herpetomonas, L = Leishmania

L-Trifluoromethyl cysteine

Biological activity profiles versus a variety of micro-organisms

Growth inhibition results for a variety of micro-organisms in the presence of varying concentrations of L-Trifluoromethionine.

Drug	T. augusta		C. fasciculata		H.m. muscanum		H.m. ingenoplastis		L. tarentolae		T. pyriforris	
Conc^{n} μg ml^{-1}	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
100	+3	+3	+	+	+	+	-	-	↑		+2	+1
50	+3	+3	+1	+1	+	+	+1	+1	↑		+3	+2
25	+3	+3	+2	+2	+	+	+3	+3	DEAD		+3	+3
10	+3	+3	+2	+2	+	+	+3	+3	Did		+3	+3
5	+3	+3	+3	+3	+1	+1	+3	+3	Not		+3	+3
2.5	+3	+3	+3	+3	+2	+2	+3	+3	Grow		+3	+3
1	+3	+3	+3	+3	+2	+2	+3	+3	↓		+3	+3
control	+3	+3	+3	+3	+3	+3	+3	+3	↓		+3	+3

Test conditions: 20μl aqueous drug solution + 180μl culture, 48h, aerobic incubation, 25°C, microtitre plate. Ref (84) 29/10/93

Drug	T. augusta		C. fasciculata		H.m. muscanum		H.m. ingenoplastis		L. tarentolae		T. pyriforris	
Conc^{n} μg ml^{-1}	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
100	↑		+	+	+	+	-	-	↑		+3	+3
50			+1	+1	+	+	-	-	↑		+3	+3
25	↑		+2	+2	+1	+1	+	+	DEAD		+3	+3
10	Over-		+2	+2	+2	+2	+	+	Did		+3	+3
5	grown		+3	+3	+2	+2	+3	+3	Not		+3	+3
2.5	↑		+3	+3	+3	+2	+3	+3	Grow		+3	+3
1			+3	+3	+3	+3	+3	+3	↓		+3	+3
control	↓		+3	+3	+3	+3	+3	+3	↓		+3	+3

Test conditions: 20μl aqueous drug solution + 180μl culture, 120h, aerobic incubation, 25°C, microtitre plate. Ref (84) 1/11/93

D-Trifluoromethyl penicillamine

Biological activity profiles versus *Trichomonas. vaginalis*

Growth inhibition results for *T. vaginalis* in the presence of varying concentrations of D-Trifluoromethyl penicillamine

Drug	D-Trifluoromethyl penicillamine		
Conc^{n} μg ml^{-1}	Rep 1	Rep 2	Rep 3
100	+2	+2	+2
10	+3	+2	+3
1	+3	+3	+3
control	+3	+3	+3

Test conditions: 20μl aqueous drug solution + 180μl culture, 24h, anaerobic incubation, 37°C, microtitre plate. Ref (84) 29/4/94

Biological activity profile versus *Trichomonas. foetus*

Growth inhibition results for *T. foetus* in the presence of varying concentrations of D-Trifluoromethyl penicillamine.

Drug	D-Trifluoromethyl penicillamine		
Conc^{n} μg ml^{-1}	Rep 1	Rep 2	Rep 3
100	+3	+3	+3
10	+3	+3	+3
1	+3	+3	+3
control	+3	+3	+3

Test conditions: 20μl aqueous drug solution + 180μl culture, 24h, anaerobic incubation, 37°C, microtitre plate. Ref (84) 29/4/94

References

1. W. Bulloch, in *The History of Bacteriology*, N.Y.: Oxford University Press, 1960; Republished, N.Y.: Dover Publications Inc. (1979)
2. R.Y. Stainer, J.L. Ingraham, M.L. Wheelis, P.R. Painter, *General Microbiology*, 5th Edn., Ch1: The begininings of Microbiology (1987)
3. T.D. Brock, *Milestones in Microbiology*, Prentice-Hall, Inc. Englewood Cliffs, N.J. (1961)
4. R.Y. Stainer, J.L. Ingraham, M.L. Wheelis, P.R. Painter, *General Microbiology*, 5th Edn., Ch33: The exploitation of Microorganisms by Humans (1987)
5. R. Hare, *The Birth of Penicillin*, G. Allan and Unwin Ltd., London (1970)
6. L. Stryer, Biochemistry, 3rd Edn., Ch25: Synthesis of Nucleotides, W.H. Freeman and Co., N.Y. (1988)
7. E. Fischer, *Chem. Ber.*, **27**, 2985 (1894)
8. J.B. Sumner, *J. Biol. Chem.*, **69**, 435 (1926)
9. J.R. Vane, *Nature* (London), New Biol. Chem., **257**, 2528 (1971)
10. I.W. Wainer, D.E. Drayers, *Drug Stereochemistry*, eds Dekker, New York (1988)
11. M. Gross, *Ann. Rep. Med. Chem.*, **25**, 323 (1990)
12. M.A. Fox and J.K. Whitesell, *Organic Chemistry*, 2nd Edn., p264 (199-)
13. E. Shaw, *The Enzymes* (edited by P. Boyer), 3rd Edn, Vol 1, p91, Academic Press New York (1970).
14. R. Rando, *Science*, **185**, 320 (1974)
15. R. Rando, *Accts. Chem. Res.*, **8**, 281, (1975)
16. A. Maycock and R. Abeles, *Accts. Chem. Res.*, **9**, 313, (1976)
17. C. Walsh, *Horizons in Biochemistry and Biophysics,* ed. by E Quagliarielle, Vol 3, p36, Addison Wesley, Reading, Mass (1977)
18. C. Walsh, *Ann. Reus., Biochem.*, **45**, 891, (1978)
19. C. Walsh, *Molecular Biology, Biochemistry and Biophysics*, Vol 32, p62, Springer-Verlag, Heidelberg (1980)
20. F. Miesowicz and K. Bloch, *J. Biol. Chem.*, **253**, 5868 (1979)

21. M. Johnston, D. Jankowski, P. Marcotte, H. Tanaka, N. Esaki, K. Soda and C. Walsh, *Biochemistry*, **18**, 4690, (1979)
22. T. Cromartie and C. Walsh, *Ibid.*, **14**, 3482 (1975)
23. J. Kollonitsch, L. Barash, F. Kahan and H. Kropp, *Nature*, **243**, 346, (1973)
24. F. Kahan, H. Kropp, H. Wooruff, J. Kollonitsch, L. Barash, N. Jensen, D. Jacobus, Abstracts, *Inter Science Conference on Antimicrobial Agents and Chemotherapy*, Abstracts 101 - 103, Washington D.C. (1975)
25. E. Wang and C. Walsh, *Biochemistry*, **17**, 1313, (1978)
26. R. Rando, *Biochemistry*, **13**, 3859, (1974)
27. T. Soper, J. Manning, P. Marcotte and C. Walsh, *J. Biol. Chem.* **252**, 1571, (1977)
28. *Organo Fluorine Chemicals and their Industrial Applications*, ed. R.E. Banks, Ellis Horwood, Chichester U.K. (1979).
29. *Preparation, Properties and Industrial Applications of Organo Fluorine Compounds*, ed. R.E. Banks, Ellis Horwood, Chichester U.K. (1982).
30. *Organo Fluorine Chemistry, Principles and Commercial Applications*, ed. R.E. Banks, B.E. Smart, and J.C. Tatlow, Plenum, New York (1993)
31. J.A. Wilkinson, *Chem. Rev.*, **92**, 505-519 (1992)
32. R. Field in *Fluorine - The First Hundred Years* (1886 - 1986), ed. R.E. Banks, DWA Sharp and J.C. Tatlow, Elsevier Sequoia, Lausanne, pp71-108 (1986)
33. R. Peters in *Carbon - Fluorine Compounds: Chemistry Biochemistry and Biological Activities*, Elsevier-Excerpta Medica-North, Holland, Amsterdam, pp55-75 (1972)
34. K.L. Kirk, *Biochemistry of Halogenated Organic Compounds*, Plenum, New York (1991)
35. W.G.M. Jones in *Organo Fluorine Chemicals and their Industrial Applications*, ed. R.E. Banks, Ellis Horwood, Chichester U.K., pp 154-168 (1979)
36. W.G.M. Jones in *Preparation, Properties and Industrial Applications of Organo Fluorine Compounds*, e.d. R.E. Banks, Ellis Horwood, Chichester U.K., pp 156-192 (1982).

37. J. Fried and E.F. Sabo, *J. Amer. Chem. Soc.*, **76**, 1455-1456 (1954)
38. C. Heidelberger in *Carbon - Fluorine Compounds: Chemistry Biochemistry and Biological Activities*, Elsevier-Excerpta Medica-North, Holland, Amsterdam (1972)
39. M.O. Meyer, D. O'Hagan *Chem. Br.*, **28**, 785 (1992)
40. K.L. Kirk, *Biochemistry of the Elemental Halogens and Inorganic Halides*, Plenum Press, New York (1991)
41. S.L. Neidleman, J. Geigert, *Biohalogenation: Principles, Basic Roles and Applications*, John Wiley and Sons, New York (1986)
42. S.W. Guy, D.R. Taves W.S. Brey Jr., *ACS Symp. Ser. 28, Amer. Chem. Soc*, Washington D.C. pp117-134 (1976)
43. P.F.V. Ward, R.J. Hall, R.A. Peters, *Nature*, 201 and 611 (1964)
44. C. Heidelberger, D. Parsons, D.C. Remy, *J. Amer. Chem. Soc.*, **84**, 3592 (1962)
45. G. Etzold, R. Hintsche, G. Kowollik, P. Langen, *Tetrahedron*, **27**, 2463, (1971)
46. P. Herdewijn, J. Balzarini, M. Baba, R. Pauwels, A.V. Aerschot, G. Janssen, E. De Clercq., *J. Med. Chem.*, **31**, 2040 (1988)
47. A. Hizi, R. Tal, M. Shaharabany, M.J. Currens, M.R. Boyd, S.H. Hughes, J.B. McMahon, *Antimicrob. Agents Chemother.*, **37**, 1037 (1993)
48. K. Holmberg and R.D. Meyer, *J. Inf. Dis.*, **18**, 179, (1986)
49. A. Bell, *Antifungal and Tropical Diseases, Chemserve Fluorine in Medicine in the 21st Century Conference, UMIST*, Conference Paper 6 (1994)
50. A.J. Cooper, *Ann. Rev. Biochem.* **52**, 87-222, (1983)
51. C.W. Tabor, H. Tabor, *Adv. Enzymol.*, **56**, 251-282, (1984)
52. A. Finkelstein N.J. Benevenga, *J. Nutr.*, **16**, 204-205 (1986)
53. R.D. Steel, N.J. Benevenga, *J. Biol. Chem.* **253**, 7844-7850 (1978)
54. W. Segal. R.L. Starkey, *J. Bacteriol.*, **98**, 908-913 (1969)
55. J. Ruiz-Herrera, R.L. Starkey, *J. Bacteriol.*, **99**, 544-551 (1969)
56. H. Tanaka, N. Esaki, K. Soda, *Biochemistry*, **16**, 100-106 (1977)
57. B.C. Lockwood, G.H. Coombs, *J., Biochem.* **279**, 675-682 (1991)

58. M. Ferchichi, D. Hemme, M. Nardi, N. Pamboukdjian, *J. Gen. Microbiol.*, **131**, 715-723 (1985)
59. K.W. Thong, G.H. Coombs, B.E. Sanderson, *Molecular and Biochemical Parisitology*, **23**, 223-231 (1987)
60. K.W. Thong, G.H. Coombs, B.E. Sanderson, *Acta. Univ. Carol. Biol.* **10**, 293-398 (1987).
61. W.T. Shults, E.N. Fountain, E,C. Lynch, *J. Am. Med. Assc.*, **211**, 2153-2154 (1970)
62. L. Zieve, W.M. Doizaki, F.J. Zieve, *J. Lab. Cli. Med.*, **83**, 16-27 (1974)
63. J.C. Walker, S. Morrel, H.H. Foster, *Am. J. Bot.*, **24**, 536-541 (1937)
64. R.J. Waller, *Toxicol. Appl. Pharmacol.*, **42**, 111-117 (1977)
65. J.I. Toohey, *Biochem. Biophys. Res. Commun.*, **78**, 1273-1280 (1977)
66. H. Kadota and Y. Ishida, *Ann. Rev. Microbiol.*, **26**, 127-138 (1972)
67. Silvey and Cady, *J. Amer. Chem. Soc.*, **72**, 3624 (1950)
68. G.A.R. Brandt, H.J. Emeléus, R.N. Hazeldine, *Organometalloidal Fluorine Compounds*, part III, 2198 (1952)
69. G.A.R. Brandt, H.J. Emeléus, R.N. Hazeldine, *Organometalloidal Fluorine Compounds*, part IV, 2549 (1952)
70. R.N. Hazeldine and J.M. Kidd, *J. Chem. Soc.*, 3219 (1953)
71. R.L. Dannley and R.G. Taborsky, *J. Org. Chem.*, **22**, 1275 (1957)
72. J.K. Kim and J.F. Bunnett, *J. Amer. Chem. Soc.*, **92**, 7463 (1970)
73. R.A. Rossi and J.F. Bunnett, *J. Amer. Chem. Soc.* **94**, 683 (1972)
74. J.F. Bunnett and X. Creary, *J. Org. Chem.*, **39**, 3173, 3611 (1974)
75. V.N. Boiko, G.M. Shchupak, L.M. Yagupol'skii, *J. Org. Chem.*, **13**, 972, (1977)
76. J.J. Lagowski, *Quart. Rev.*, **13**, 233 (1959)
77. L. Pauling, *General Chemistry*, Russian Translation, *Izd. Mir*, 168 (1974)
78. M.E. Houston and J.F. Honek, *J. Chem. Soc. Chem. Comm.*, 761 (1989)
79. A.F. Janzen, P.M.C. Wang, A.E. Lemire, *J. Fluorine Chem.*, **22**, 557, (1983)
80. V. Soloshonok, V. Kukhar, Y. Pustovit, V. Nazaretian, *Synlett*, 657 (1992)
81. M. Johnston, R. Raines, C. Walsh, R.A. Firestone, *J. Amer. Chem. Soc.*, **102**, 4241 (1980)

82. R. Tang and K. Mislow, *J. Amer. Chem. Soc.*, **9**, 2100 (1970)
83. J. Kollintsch and L. Barash, *J. Amer. Chem. Soc.*, **98**, 5591, (1976)
84. G.H. Coombs, unpublished work, Dept of Infection and Immunity, Institute of Biomedical and Life Sciences, University of Glasgow (1998)
85. T.A. Alston and H.A. Bright, *Biochemical Pharmacology*, **32**, 947, (1983)
86. S. Black and N.G. Wright,
87. Fieser and Fieser, *Reagents for Organic Chemistry Preparation*,
88. R.A. Turner and R. Connor, Ibid., **69**, 1009, (1947)
89. R. Katakai, *J. Chem Soc., Perkin Trans 1.*, 2249 (1987)
90. F.A. Cotton and G. Wilkinson, *Basic Inorganic Chemistry*, Wiley International Ed^{n}, p10, (1976)
91. N. Kharasch, *Organic Sulfur Compounds*, Vol 1, N. Kharasch, Ed., Pergamon Press, Inc., New York, p375-396, (1961)
92. I.B. Douglass, *Organic Sulfur Compounds*, Vol 1, N. Kharasch, Ed., Pergamon Press, Inc., New York, p350-360 (1961)
93. F.A. Drahowzal, *Organic Sulfur Compounds*, Vol 1, N. Kharasch, Ed., Pergamon Press, Inc., New York, p361-374 (1961)
94. N. Kharasch, Z.S.Ariyan and A.J. Havlik, *Quart. Rept. Sulfur Chem.*,1. 93 (1966)
95. C.M. Buess and N. Kharasch, *J. Amer. Chem. Soc.*, **72**, 3529 (1950)
96. W.L. Orr and N. Kharasch, Ibid., **75**, 6035 (1953)
97. D.R. Hogg and N. Kharasch, Ibid., **78**, 2728 (1956)
98. D.J. Cram, *J. Amer. Chem. Soc.*, **71**, 3383 (1949)
99. N. Kharasch and A.J. Havlik, Ibid., **74**, 3734 (1953)
100. A.J. Havlik and N. Kharasch, Ibid., **78**, 1207, (1956)
101. G.H. Schmid and V.M. Szismadia, Can. J. Chem., **44**, 1338 (1966)
102. W.H. Mueller and P.E. Butler, J. Amer. Chem. Soc., **90**, 2075 (1968)
103. G. Resnati, *Tetrahedron*, **49**, 9386, 1993
104. L. Ruzicka, *Agnew. Chem.*, Int. Ed. Engl., 29, 1320 (1990)
105. A. Haas, *Gmelins's Handbuch der anorganischen Chemie*, 8th Ed., **9** (1973)
106. A. Haas and U.Niemann, *Advances in Inorganic Chemistry*, **16**, 143 (1976)

107. K.A. Petrov and A.A. Neimysheva, *Zh. Obshch. Khim.*, **29**, 3401 (1959)

108. A.Haas, M. Lieb and Yuanfa Zhang, *Journal of Fluorine Chemistry*, **30**, 203 (1985)

109. L.A. Caprino and G.Y. Han, *J. Org. Chem.*, **37**, 3404 (1972)

110. J. Blake, *Int. J. Pept. Protein Res.*, **13**, 418 (1979)

111. J.P. Tam, Tai-Wai Wong, M.W. Rieman, Foe-s Tjoeng and R.B. Merrifield, *Tetrahedron Lett.*, 4033 (1979)

112. K. Ishihara, M. Kubota, H. Kurihara and H. Yamamoto, *J. Amer. Chem. Soc.*, **117**, 4413 (1995)

113. U.S. Pat. 2000932; Zbl. I, 875, (1936); British patent. 454577; Zbl. I, 1013, (1937)

114. N.N. Yarovenko, S.P. Motornyl, A.S. Vasil'eva and T.P. Gershzon, *J. of Gen Chem. U.S.S.R.*, **29**, 2129 (1959)

115. H. Lecher and F. Holschneider, *Ber.*, **57**, 755 (1924)